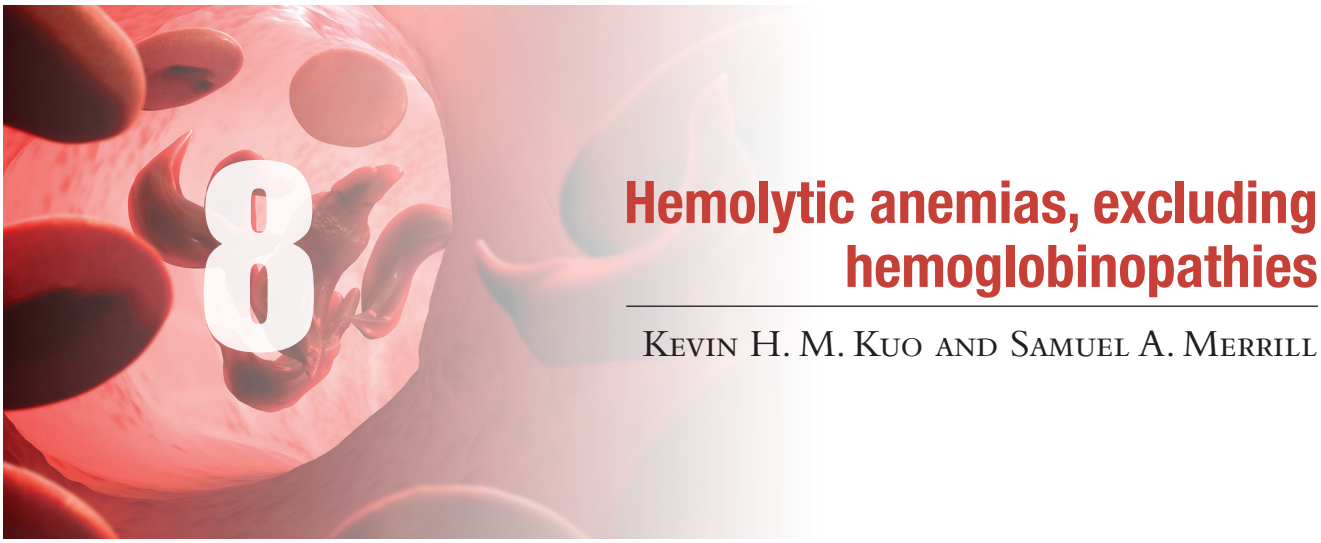

Hemolytic anemias, excluding hemoglobinopathies

Kevin H. M. Kuo and Samuel A. Merrill

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Introduction

Hemolysis is the accelerated destruction of red blood cells (RBCs), leading to decreased RBC survival. The bone marrow's response to hemolysis is increased erythropoiesis, reflected by reticulocytosis. If the rate of hemolysis is modest and the bone marrow is able to completely compensate for the decreased RBC life span, the hemoglobin concentration may be normal; this is called *fully compensated hemolysis*. Anemia occurs if the bone marrow is unable to completely compensate for hemolysis (called *incompletely compensated hemolysis*). Thus, a hemoglobin value within the normal range does not necessary denote the absence of hemolysis.

Clinically, hemolytic anemia produces variable degrees of fatigue, pallor, jaundice, and gallstones. Splenomegaly occurs in some conditions. The complete blood count may show anemia and reticulocytosis, but this depends on the acuity and severity of hemolysis and the degree of compensation by the bone marrow. Secondary chemical changes include indirect hyperbilirubinemia, increased urobilinogen excretion, and elevated lactate dehydrogenase (LDH). Decreased serum haptoglobin levels are common and increased plasma-free hemoglobin may also be detected. Because free hemoglobin scavenges nitric oxide (NO) and release of RBC arginase impairs NO bioavailability, erectile dysfunction, esophageal spasm, renal insufficiency, or vascular sequelae such as nonhealing skin ulcers and pulmonary hypertension can occur in chronic hemolytic anemia (Figure 8-1). Chronic intravascular hemolysis produces hemosiderinuria, and chronic extravascular hemolysis increases the risk of pigmented (bilirubinate) gallstones. Some hemolytic conditions are also associated with increased risk of thrombosis, because of abnormal RBC properties, increased plasma concentrations of microparticles, release of cell-free hemoglobin, increased reactive oxygen species, and endothelial dysfunction (Figure 8-1). Iron overload is observed in 33% to 71% of congenital hemolytic anemias even in the absence of chronic transfusion, the mechanism of which has not been fully elucidated.

The hemolytic anemias can be classified and approached in different yet complementary ways (Table 8-1). They can be inherited (eg, sickle cell disease or hereditary spherocytosis) or acquired (eg, autoimmune or microangiopathic). They can sometimes be distinguished via a thorough history, including the tempo of chronic hemolysis (episodic versus chronic), time of onset of

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Off-label drug use: Azathioprine, bortezomib, chlorambucil, cyclophosphamide, cyclosporine, danazol, eculizumab, erythropoietin, intravenous immunoglobulin, mycophenolate mofetil, and rituximab in the treatment of autoimmune hemolytic anemia. Sutimlimab in the treatment of cold agglutinin disease. Eculizumab in the treatment of Shiga toxin–mediated thrombotic microangiopathy (TMA) and drug-induced TMA.

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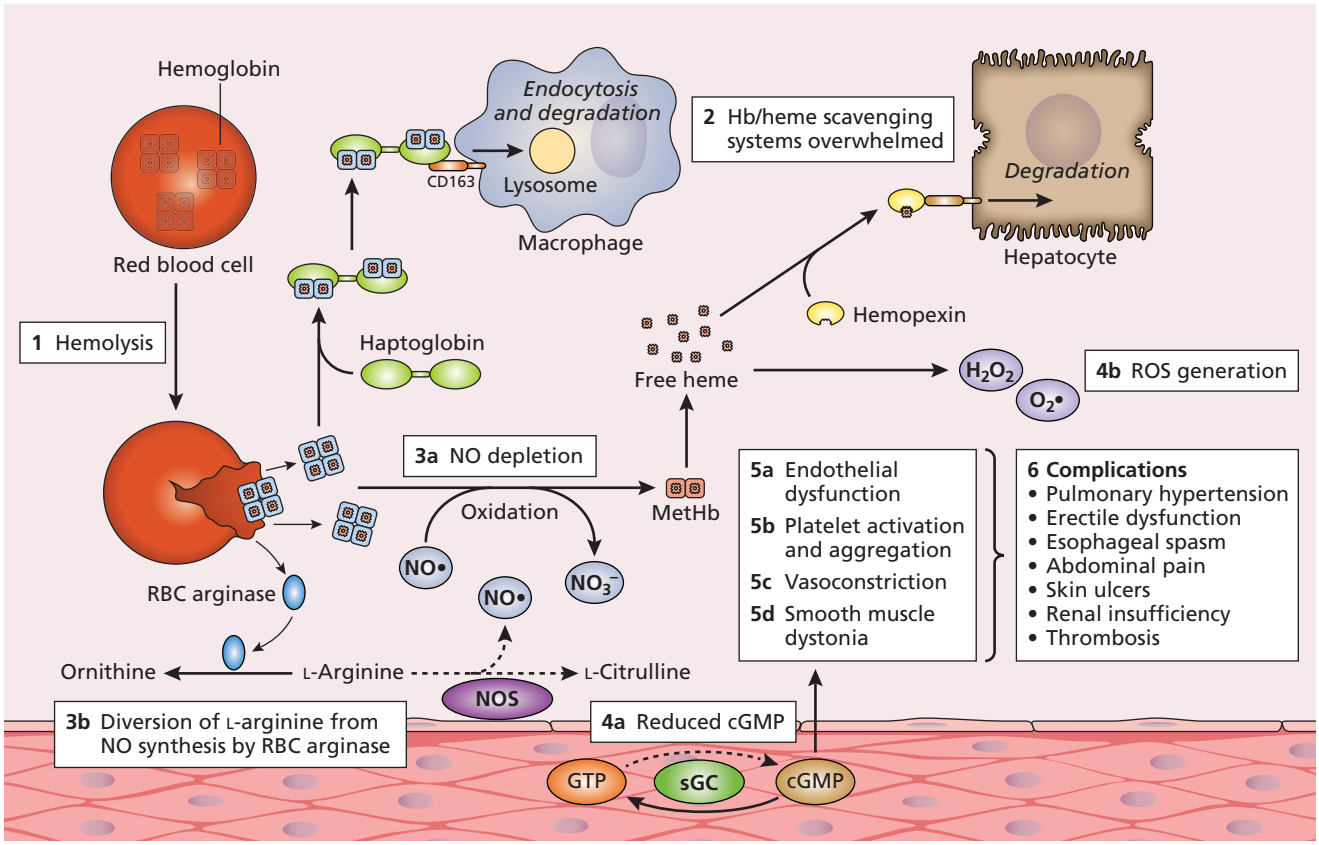

Figure 8-1 The pathophysiology of complications from chronic intravascular hemolysis. cGMP, cyclic guanosine monophosphate; GTP, guanosine triphosphate; Hb, hemoglobin; MetHb, methemoglobin; NOS, nitric oxide synthase; ROS, reactive oxygen species; sGC, soluble guanylyl cyclase. Adapted from Schechter AN, *Blood*. 2008;112(10):3927-3938; Gladwin MT, Ofori-Acquah SF, *Blood*. 2014;123(24):3689-3690; and Schaer DJ et al, *Front Physiol*. 2014;5:415.

hemolytic complications, prior blood counts and transfusions, antecedent factors leading to the hemolytic episode, and the kinetics in the fall in hemoglobin.

Alternatively, they can be characterized by the anatomic site of RBC destruction: extravascular or intravascular. Extravascular hemolysis, in which erythrocyte destruction occurs by macrophages in the liver and spleen, is more common. Intravascular hemolysis refers to RBC destruction occurring primarily within blood vessels. The distinction between intravascular and extravascular hemolysis is not absolute because both occur simultaneously (at least to some degree) in the same patient, and the manifestations of both can overlap. The site of RBC destruction in different conditions can be conceptualized to occur in a spectrum between pure intravascular and pure extravascular hemolysis. Some hemolytic anemias are predominantly intravascular (eg, paroxysmal nocturnal hemoglobinuria [PNH]), and some are predominantly extravascular (eg, hereditary spherocytosis [HS]). Others have substantial components of both, such as sickle cell disease. While high LDH and low haptoglobin levels are commonly seen in both intravascular and extravascular hemolysis, these values can be normal in extravascular hemolysis. The presence of hemoglobinuria or hemosiderinuria suggests intravascular hemolysis.

Table 8-1 Methods of classification of hemolytic anemias

Classification	Example
Inheritance	
Inherited	Sickle cell anemia
Acquired	Autoimmune hemolytic anemia
Site of red blood cell (RBC) destruction	
Intravascular	Paroxysmal nocturnal hemoglobinuria
Extravascular	Hereditary spherocytosis
Origin of RBC damage	
Intrinsic	Pyruvate kinase deficiency
Extrinsic	Thrombotic thrombocytopenic purpura

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The hemolytic anemias can also be classified according to whether the cause of hemolysis is intrinsic or extrinsic to the RBC. Intrinsic causes of hemolysis include abnormalities in hemoglobin structure or function, the RBC membrane, or RBC metabolism (cytosolic enzymes). Extrinsic causes may be caused by an RBC-directed antibody, a disordered vasculature, or the presence of infecting organisms or toxins. In general, intrinsic causes of hemolysis are inherited and extrinsic causes are acquired, but there are notable exceptions. For example, PNH is an acquired intrinsic RBC disorder, and congenital thrombotic thrombocytopenia purpura (TTP) is an inherited cause of extrinsic hemolysis. Peripheral blood film may provide morphologic clues to the diagnosis.

In this chapter, hemolytic anemias are divided into those that are caused by intrinsic or extrinsic abnormalities of the RBC. Hemoglobinopathies are covered in Chapter 7.

Hemolysis caused by intrinsic abnormalities of the RBC

Intrinsic causes of hemolysis include abnormalities of hemoglobin structure or function, the RBC membrane, or RBC metabolism (cytosolic enzymes). Most intrinsic forms of hemolysis are inherited conditions. The geographic association between malaria endemicity and the frequency of a number of congenital hemolytic anemias, including glucose-6-phosphate dehydrogenase (G6PD) deficiency, pyruvate kinase (PK) deficiency (PKD), hereditary elliptocytosis (HE), and dehydrating hereditary stomatocytosis (DHSt), have prompted the malaria hypothesis, where the heterozygous state of these disorders confers resistance against malarial infection or cerebral malaria.

Abnormalities of the RBC membrane

CLINICAL CASE

A 36-year-old woman is referred for evaluation of moderate anemia since childhood; she has been prescribed iron intermittently. She is asymptomatic aside from mild fatigue. Her past history is significant for intermittent jaundice and a cholecystectomy for gallstones at age 22 years. She takes no medications. Her paternal cousin and paternal aunt also have anemia and jaundice. Her examination is significant for mild splenomegaly. Today's hemoglobin is 9.0 g/dL (baseline 8.0 and 11.0 g/dL), mean corpuscular volume (MCV) is 94 fL, and mean corpuscular hemoglobin concentration (MCHC) is 38.0 g/dL. The absolute reticulocyte count is 252×10^9/L. Peripheral blood film reveals numerous spherocytes.

HS and hereditary elliptocytosis/hereditary pyropoikilocytosis (HE/HPP) are a heterogeneous group of hemolytic disorders with a wide spectrum of clinical manifestations. HS and HE/HPP are characterized by abnormal shape and flexibility of RBCs because of a deficiency or dysfunction of 1 or more of the membrane proteins, while overhydrated (OHSt), dehydrated hereditary stomatocytosis (DHSt; also known as hereditary xerocytosis [HX]), and cryohydrocytosis affectation permeability of the RBC membrane. Multiple genetic abnormalities, including deletions, point mutations, and defective mRNA processing, have been identified as underlying causes.

RBC membrane protein composition and assembly

An RBC in circulation is a dynamic structure that continuously deforms and flexes under shear stress. Its strength and flexibility are derived from the RBC membrane, made up of a relatively fluid phospholipid-cholesterol lipid bilayer intercalated by transmembrane protein complexes stabilized by attachment to a cytoskeleton. The skeleton is organized into a hexagonal lattice, with 2 α- and β-spectrin heterodimers arranged in an antiparallel fashion to form tetramers making up the sides of each triangle unit in the hexagonal lattice (Figure 8-2a). At each corner of the triangle is the junctional complex, composed of actin oligomer capped by adducin and tropomodulin, which then connects to the spectrin tetramer via protein 4.1R. This horizontal interaction of α- and β-spectrin and spectrin-actin-protein 4.1R forms the cytoskeleton, which regulates membrane deformability and mechanical integrity. The cytoskeleton is then attached to the lipid bilayer via vertical interaction with the transmembrane protein complexes, composed of integral membrane proteins band 3 and Rh-associated glycophorin (RhAG) and peripheral membrane proteins ankyrin and band 4.2 (Figure 8-2b). In addition, ion channels such as PIEZO1 and KCNN4 that traverse the lipid bilayer regulate intracellular sodium and potassium content. Thus, defect in horizontal interaction will lead to reduced membrane integrity and cell fragmentation, whereas loss of vertical interaction results in loss of membrane surface area, and inability to regulate intracellular sodium and potassium content will lead to cellular dehydration or overhydration, increasing membrane rigidity. These defective RBCs are destroyed in circulation or the spleen as they are unable to withstand the shear stress and reversibly deform while traversing through the capillaries and the interendothelial slits of the splenic sinusoids. Consequently, for the purposes of conceptualization, some in the field describes HS as tending to involve defects in proteins responsible for the "vertical" interaction whereas HE/HPP tend to be caused by

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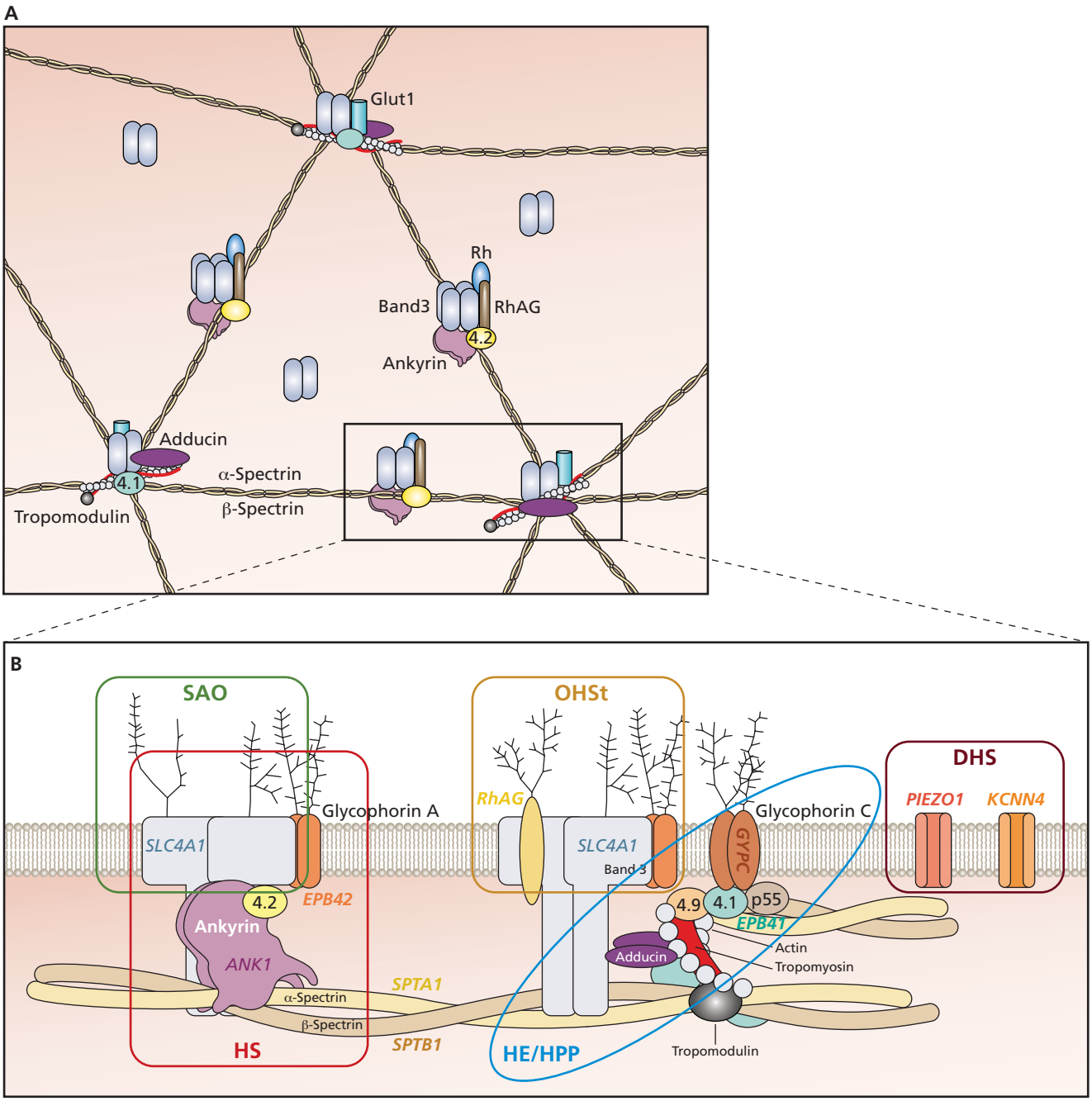

Figure 8-2 Red blood cell membrane cytoskeletal structure and the associated membranopathies, with the corresponding genes responsible encircled. (a) The quasihexagonal lattice of the RBC cytoskeleton, with the a- and b-spectrin heterodimers, associate head-to-head to form the spectrin tetramers that make the sides of each triangular unit in the hexagon. The junctional complex at the corner of each triangle is formed by an actin oligomer, with a length guided by tropomyosin and capped by adducin and tropomodulin. Protein 4.1R enables the "horizontal" actin-spectrin association. The transmembrane protein complexes containing the integral membrane proteins band 3 and Rh-associated glycoprotein (RhAG) and the peripheral membrane proteins ankyrin and band 4.2 provide "vertical" interaction between the cytoskeleton and the lipid bilayer. (b) Mutations from different cytoskeletal genes can give rise to the same phenotype, while certain proteins, such as band 3, can give rise to different phenotypes depending on the location and nature of the mutation. Note that HS and HE/HPP are caused by defects in protein-protein interactions in the vertical and horizontal axes, respectively. DHS, dehydrated hereditary stomatocytosis. Adapted from Risinger M, Kalfa TA, *Blood*. 2020;136(11):1250-1261 and Liem R, Gallagher PG, *Drug Discov Today Dis Mech*. 2005;2(4):539-45, with permission from the publishers.

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defects in proteins responsible for the "horizontal" interactions, while keeping in mind that this is a generalization and not an absolute correlation.

Hereditary spherocytosis

HS is most common in individuals of northern European descent with a prevalence of 1 in 1000 to 2500. The prevalence of a clinically recognized disorder is much lower given the variable penetrance. Two-thirds of cases are inherited in an autosomal dominant fashion, represented by heterozygous mutations in ankyrin (*ANK1*), band 3 (*SLC4A1*), and β-spectrin (*SPTB*), whereas 10% to 15% of the cases are caused by biallelic defects in band 4.2 (*EPB42*), α-spectrin (*SPTA*), and ankyrin (*ANK1*) leading to an autosomal recessive inheritance pattern (Table 8-2). The rest are de novo mutations, most commonly found in *ANK1*. The HS syndromes generally are caused by private mutations unique to each kindred. HS is characterized by spherocytic, osmotically fragile RBCs and is both clinically and genetically heterogeneous (Figure 8-3a).

Pathophysiology

The pathophysiology of HS generally involves aberrant interactions between the cytoskeleton and the lipid bilayer (vertical interactions). A common epiphenomenon in HS RBCs is a varying degree of spectrin loss, which is usually caused by a defect in one of the membrane proteins involved in the attachment of spectrin to the membrane rather than a primary defect in the spectrin molecule itself. Spectrin is the major protein of the skeleton and forms a nearly monomolecular submembrane layer that covers most of the inner-membrane surface; therefore, the density of this skeletal layer in HS erythrocytes is reduced. Consequently, the lipid bilayer is destabilized, leading to loss of membrane lipid and thus surface area through microvesiculation. The result of these changes is a progressively spheroidal RBC. The inherent reduced deformability of spherocytes makes it difficult for them to traverse the unique constraining apertures that characterize splenic vascular walls. The spleen "conditions" RBCs, enhancing membrane loss. Retained and further damaged by the hypoxic and acidic environment in the spleen, they are ultimately prematurely destroyed.

Clinical manifestations

The clinical expression ranges from an asymptomatic and often undiagnosed condition with nearly normal hemoglobin levels (compensated hemolysis) to severe hemolysis and anemia. Patients with mild HS have a relatively uneventful course, although some may develop pigmented gallstones in childhood or adult life. Mildly anemic patients may be diagnosed later in life as adults during evaluation for unrelated conditions. Patients with moderately severe disease may present with several additional complications. Aplastic crisis, which may be the initial presentation for some patients, may require urgent attention. The cause of aplastic crisis is human parvovirus infection, which produces selective suppression of erythropoiesis, resulting in reticulocytopenia and inability to compensate for ongoing RBC destruction. In contrast, the hyperhemolytic crisis is characterized by accelerated hemolysis, leading to increased jaundice and splenic enlargement, which is a common problem in children. Because all HS patients are liable to present with neonatal jaundice, they require appropriate monitoring and preemptive phototherapy (rarely exchange transfusion) to avoid kernicterus. The exacerbation of neonatal jaundice in HS and HE/HPP patients was thought to be caused by the abundance of fetal hemoglobin (HbF) and diminished binding of 2,3-diphosphoglyceric acid (DPG) to HbF rendering the RBCs susceptible to fragmentation. Other complications include the rare megaloblastic crisis secondary to acquired folic acid deficiency, usually associated with high-demand situations such as pregnancy. Leg ulcerations have been rarely reported. Patients with severe hemolysis and resulting expansion of the erythroid compartment in the bone marrow can develop maxillary hyperplasia interfering with dentition or extramedullary hematopoietic masses that may

Table 8-2 Defects of red blood cell membrane proteins in hereditary spherocytosis, elliptocytosis, and pyropoikilocytosis

Class of defect	Hereditary spherocytosis	Hereditary elliptocytosis and pyropoikilocytosis
Protein deficiency (gene)	Spectrin (*SPTA1, SPTB1*)	Spectrin† (*SPTA1, SPTB1*)
	Ankyrin★ (*ANK1*)	Protein 4.1 (*EPB41*)
	Band 3 (*SLC4A1*)	Glycophorin C (*GYPC*)
	Protein 4.2 (*EPB42*)	
Protein dysfunction	β-spectrin abnormality affects β-spectrin–protein 4.1 interaction★	Defective spectrin dimer self-association caused by spectrin mutations
		Protein 4.1 abnormality affects β-spectrin–protein 4.1 interaction

★Red blood cells of these patients are also partially deficient in spectrin.

†Seen in patients with hereditary pyropoikilocytosis in cases in which it coexists with a spectrin mutation that affects spectrin self-association.

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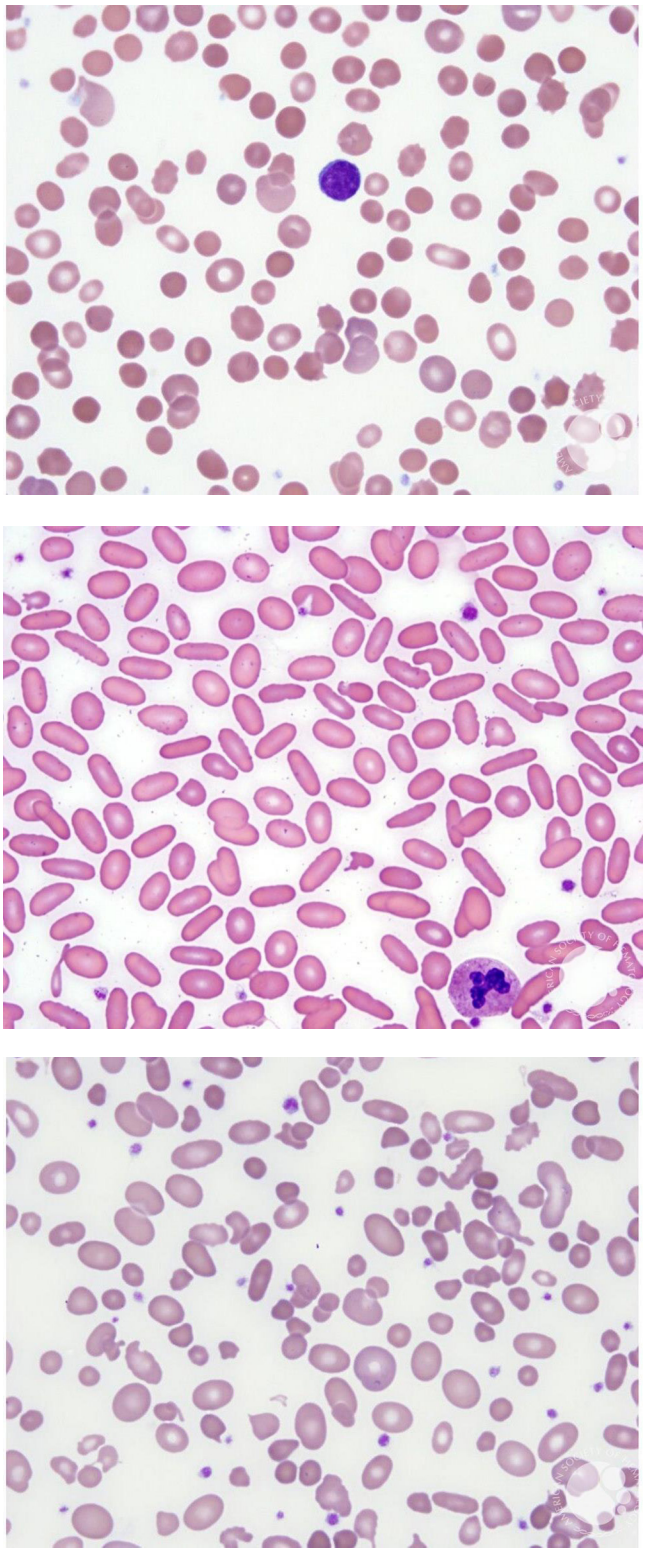

Figure 8-3 Peripheral blood findings in inherited disorders of the red cell membrane. (Top) Numerous spherocytes; (middle) numerous elliptocytes and a rod-shaped cell; (bottom) marked poikilocytosis. Source: ASH Image Bank/Teresa Scordino (images 00060308, 00060270, 00060300).

mimic malignancy. Iron overload is a chronic complication of HS, even in non-transfusion-dependent patients. A ferritin >500 mg/l and transferrin saturation >60% is the best combination for predicting iron overload in these patients (liver iron concentration >4 mg Fe/g dry weight). Patients may manifest a variety of issues attributable to splenomegaly, including early satiety, left upper quadrant fullness, and hypersplenism. HS may be diagnosed in the neonatal period based on a positive family history or marked jaundice. The diagnosis also should be considered in patients of all ages with intermittent jaundice, mild refractory anemia, or splenomegaly. Rare associated syndromes suggest that mutant RBC membrane proteins may reside in other tissues. For example, distal renal tubular acidosis may occur in HS patients harboring mutant band 3 (the anion channel protein).

Rarely, HS can present as hydrops fetalis caused by either homozygous *SLC4A1* or biallelic null *SPTA1* mutations. The fetus may be rescued with intrauterine transfusion or premature delivery to provide early transfusion support. These patients also have reticulocytopenia and splenectomy does not significantly improve the anemia.

Laboratory evaluation

In addition to the usual laboratory abnormalities indicating hemolysis, the principal diagnostic feature is the identification of spherocytes on the peripheral blood film (Figure 8-3a). The extent of spherocytosis is variable, and in mild cases, it may present as stomatocytosis and be missed even by the experienced clinician. Additional morphologic abnormalities, including cells with membrane extrusions and elliptocytes, may be observed. The RBC indices may provide a clue, with an increase in the MCHC (caused by cellular dehydration), but usually a normal MCV. Review of the complete blood count, reticulocyte count, and peripheral film from family members may prove helpful. The differential diagnosis for spherocytes includes autoimmune and drug-induced hemolytic anemia, so exclusion of these causes and a direct antiglobulin test (DAT) should be performed as part of the evaluation when the family history is negative. Likewise, HS should be considered in the differential diagnosis of DAT-negative hemolytic anemia.

Several specialized diagnostic tests are available to diagnose and distinguish different membranopathies. Eosin-5′-maleimide (EMA) binding assay relies on EMA binding to band 3 on RBCs; a reduction in binding, measured by fluorescence intensity, corresponds to a quantitative reduction in erythrocyte band 3 or the band 3 complex. The cryohemolysis test uses an increased susceptibility of HS

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red cells to rapid cooling from 37°C to 0°C in hypertonic conditions. Osmotic gradient ektacytometry measures deformability of whole RBCs under constant shear stress as a function of osmolality using a laser-diffraction viscometer, and it can help differentiate HS from HE/HPP and stomatocytosis. The osmotic fragility test using increasingly hypotonic saline solutions supports the diagnosis with the finding of increased RBC lysis compared with normal RBCs. Sensitivity of the test is enhanced by 24-hour incubation at 37°C, but mild cases still can be missed by the test. EMA binding assay and cryohemolysis test are the recommended screening test for cases that are equivocal. EMA binding assay has better specificity and sensitivity than osmotic fragility test and is comparable to ektacytometry. The advent of next-generation sequencing (NGS) has been helpful in isolating the causative gene mutation in HS (see section on NGS).

Treatment

As with other hemolytic anemias, folic acid supplementation should be considered for patients with severe anemia, even though overt folic acid deficiency rarely is encountered in the industrial nations because of supplementation in grain products. Patients need to be aware of the signs and symptoms of aplastic and hyperhemolytic crises to seek prompt medical attention. The definitive treatment of HS is splenectomy, which ameliorates the hemolytic anemia in almost all patients, although the underlying intrinsic defect of the circulating RBCs is not altered. Dehydrated hereditary stomatocytosis (DHSt) must be ruled out prior to splenectomy as it is contraindicated in DHSt (see section on hereditary stomatocytosis). In rare patients with HS and severe hemolysis, splenectomy markedly diminishes the hemolytic rate but may not fully correct the anemia. Clinical trial data are not available to provide guidelines for making the decision to recommend splenectomy. Thus, the indications for splenectomy are somewhat controversial, but the prevailing view (eg, Iolascon et al) advocates splenectomy for patients with symptomatic hemolytic anemia or its complications. Additional considerations for splenectomy in the pediatric population include failure to thrive, recurrent hyperhemolytic episodes, transfusion dependency, or complications of chronic anemia, including a hypermetabolic state. The laparoscopic technique often is preferred to open splenectomy. Accessory spleens are common, so a thorough search should be performed at the time of splenectomy. The patient should receive pneumococcal, *Haemophilus influenzae* type b, and meningococcal vaccines before the procedure, and pediatric patients usually receive prophylactic penicillin for at least several years thereafter to reduce the risk of bacterial sepsis. Thromboembolic events may occur following splenectomy, although data are limited. Because of the increased frequency of postsplenectomy infections in young children, splenectomy should not be performed before the age of 5 years except in patients with particularly severe disease. Partial splenectomy has been advocated to resolve the anemia of HS yet maintain some residual splenic phagocytic function. Long-term results of partial splenectomy (4 to 6 years) in small observational studies are promising, but the spleen may increase in size and the hemoglobin concentration may fall after splenectomy. Markers of splenic function indicate variable degrees of residual activity, but postoperative penicillin is recommended. Iron overload can be treated with iron chelation or phlebotomy but must be weighed against the side effect of iron chelation (see Tables 5-4 and 5-5), the individual's ability to tolerate phlebotomy, and potential stimulation or exacerbation of extramedullary hematopoiesis from phlebotomy.

CLINICAL CASE *(continued)*

The patient presented in this section and her father are found to have HS. Her father remains asymptomatic. It is not uncommon for the diagnosis to be made in adulthood, as patients with mild or moderate disease are often well-compensated. An elevated reticulocyte count, elevated MCHC, intermittent jaundice, history of gallstones, a negative DAT, and spherocytes on peripheral film all support the diagnosis. Genetic testing can be performed to confirm the presence of a HS-associated mutation. Family members should be evaluated for anemia.

KEY POINTS

- HS is the most common inherited hemolytic anemia of individuals from Northern Europe.
- Abnormalities in ankyrin, spectrin, band 3, and protein 4.2 ("vertical interactions") that result in a reduction in the quantity of spectrin account for the RBC membrane loss characteristic of HS.
- HS should be suspected in cases of DAT-negative hemolytic anemia when spherocytes are identified on the peripheral blood film. A positive family history is supportive of the diagnosis.
- Clinical manifestations of HS vary from a lack of symptoms to severe hemolysis.
- Splenectomy decreases hemolysis and reduces gallstone formation but should be balanced against the risk of postsplenectomy thrombosis, infection, and iron overload and thus reserved for symptomatic patients.

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Hereditary elliptocytosis and hereditary pyropoikilocytosis

The clinical presentation, inheritance, and alteration in RBC shape and physical properties and the underlying molecular defects are considerably more heterogeneous in HE/HPP than in HS. Most patients with HE are asymptomatic, with little to no hemolytic anemia, and their RBCs appear as biconcave elliptocytes (Figure 8-3b). Patients with HPP have rod-shaped cells, poikilocytes, and fragments (Figure 8-3b). Patients with Southeast Asian ovalocytosis (SAO) have a unique spoon-shaped erythrocyte morphology. In its heterozygous state, SAO newborns frequently present with neonatal jaundice but hemolysis resolves by 3 years old. Homozygous SAO result in hydrops fetalis unless salvaged with intrauterine transfusion. These patients become lifelong transfusion-dependent, with dyserythropoietic anemia and distal renal tubular acidosis. In most cases, the inheritance of HE and SAO is autosomal dominant while HPP is recessively inherited (Figure 8-3c). Some HE/HPP syndromes are caused by specific mutations in individuals from similar locales (eg, Melanesian elliptocytosis), suggesting a founder effect. Clinical manifestations range from asymptomatic carrier state to severe transfusion-dependent hemolytic anemia with poikilocytosis and erythrocyte fragmentation.

Pathophysiology

The underlying defects involve horizontal interactions between proteins of the membrane skeleton, especially spectrin-spectrin and spectrin–protein 4.1R interactions. These defects weaken the cytoskeleton. Under the influence of shear stress in the microcirculation, the cells progressively lose the ability to regain the normal disc shape and are stabilized in the elliptocytic or poikilocytic shape. In severely affected patients, the weakening of the cytoskeleton grossly diminishes membrane stability, leading to RBC fragmentation.

Different underlying molecular defects have been identified in common HE, consistent with the heterogeneous nature of the disorder (Table 8-2). In the majority of cases, patients have mutant α- or β-spectrin, resulting in defective self-association and an increased percentage of spectrin heterodimer in the membrane. A partial or complete absence or dysfunction of protein 4.1R (*EPB41*) occurs in some patients with missense and deletion mutations. Patients with HPP appear to be compound heterozygotes with the 2 *SPTA1* mutations laying in trans: one *SPTA1* HE-casing mutation and the hypomorphic α^{LELY} allele that reduces α-spectrin production by half. Since α-spectrin is normally produced in excess, individuals homozygous for α^{LELY} allele are asymptomatic, so one parent of patients with HPP is asymptomatic while the other has mild hemolytic anemia. SAO is caused by heterozygous deletion of 27 base pairs in *SLC4A1* encoding 9 amino acids at the junction of N-terminal cytoplasmic domain and the transmembrane domain of band 3.

Clinical manifestations, laboratory evaluation, and treatment

HE/HPP must be differentiated from a variety of other conditions in which elliptocytes and poikilocytes are commonly found on the peripheral blood film, including iron deficiency, thalassemia, megaloblastic anemia, myelofibrosis, and myelodysplasia. As opposed to HE/HPP, however, the percentage of elliptocytes in these other conditions usually does not exceed 60%. The presence of elliptocytes and evidence of dominant inheritance of elliptocytosis in other family members differentiate HE/HPP from the other conditions. Whereas most patients with common HE/HPP are asymptomatic, occasional patients who are homozygotes or compound heterozygotes for 1 or 2 molecular defects have more severe hemolytic disease. African American neonates with common HE may have severe hemolysis, with striking RBC abnormalities similar to HPP, which abates during the initial months of life. Approximately 10% of HE patients and all HPP patients have mild-to-moderate anemia with clinical features of pallor, jaundice, anemia, and gallstones. The most severe form of elliptocytosis, HPP, is characterized by a striking micropoikilospherocytosis and fragmentation with some elliptocytes. A markedly low MCV, typically in the range of 50 to 60 fL, may be observed. In HPP, RBCs are thermally unstable and fragment at temperatures of 46°C to 48°C, reflecting the presence of mutant spectrin in the cells. Additional specialized laboratory investigation includes separation of solubilized membrane proteins by polyacrylamide gel electrophoresis, which may reveal either an abnormally migrating spectrin or a deficiency or abnormal migration of protein 4.1. An increased fraction of unassembled dimeric spectrin can be detected by electrophoresis of RBC membrane extracts under nondenaturing conditions. NGS can been helpful in isolating the causative gene mutation in HS (see section on NGS).

Treatment is not necessary for most individuals with HE/HPP. Splenectomy may be of benefit for patients with symptomatic hemolytic anemia or its complications (see previous discussion of splenectomy for HS).

Stomatocytosis

Stomatocytes have a wide transverse slit or stoma toward the center of the RBC (Figure 8-4). A few stomatocytes (between 3% and 5%) are found on blood films of

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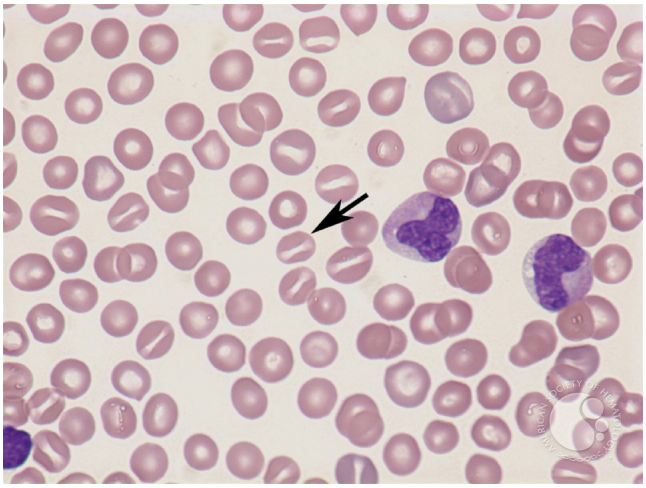

Figure 8-4 Stomatocytes. Source: ASH Image Bank/Peter Maslak (image 00003993).

healthy individuals. Several inherited and acquired disorders are associated with stomatocytosis. Stomatocytes also may occur as an artifact on peripheral blood film. Acquired stomatocytosis can be seen in acute alcoholism and hepatobiliary disease (although target cells are more common) and occasionally in malignant neoplasms and cardiovascular disorders. The inherited forms are associated with abnormalities in erythrocyte cation permeability and volume, which is either increased (OHSt), decreased (DHSt), or near normal. Like other hereditary membranopathies, genetic and clinical manifestations are highly heterogeneous. Hereditary stomatocytosis can be classified into syndromic and nonsyndromic forms. Syndromic forms of hereditary stomatocytosis are rare, and they include (a) stomatin-deficient cryohydrocytosis with mental retardation, seizures, spastic rigidity, ataxia, and hepatosplenomegaly, caused by mutations in the *SLC2A1* gene; and (b) phytosterolemia nonleaky stomatocytosis with macrothrombocytopenia, where mutations in the *ABCG5* or *ABCG8* genes lead to increased absorption and decreased excretion of sterols, resulting in xanthelasmas, accelerated atherosclerosis, severe hypercholesterolemia, abnormal incorporation of sterols into RBC membrane.

Dehydrated and overhydrated hereditary stomatocytosis

Normally, as the RBC traverse the narrow capillaries, shear stress and pressure on the RBC activates PIEZO1 causing Ca^{2+} influx, and in turn activates KCNN4 to allow K^+ efflux, drawing water out of the RBC. The resultant temporary loss in cell volume facilitates its passage through vessels 1/3 of the RBC size. Mutations in *PIEZO1* and *KCNN4* genes leading to constitutive defect in the channel activity, response to osmotic stress, or membrane trafficking can result in nonsyndromic DHSt with predominantly intravascular hemolysis. Patients with *PIEZO1*-associated DHSt typically present in adulthood with splenomegaly and/or gallstones, and not infrequently hepatic and/or cardiac iron overload, even in the absence of transfusion. PIEZO1's role in the regulation of iron metabolism has been shown both in *in vitro* hepatic model and DHSt mice model. Patients may have a history of transient perinatal edema or hydrops fetalis. RBC indices show high MCV, MCH, MCHC, and reticulocyte counts. Peripheral blood films typically contain stomatocytes, target cells, and occasionally dense cells. In majority of the cases, EMA testing is normal, but ektacytometry profile shows left shift (indicating dehydration). Given that DHSt can present as fully compensated hemolysis or mild anemia, it may be underdiagnosed and may be as common as 1 in 8000. Of significant clinical importance is the recognition that patients with *PIEZO1*-associated DHSt have a very high risk of developing thrombotic events after splenectomy. Therefore, splenectomy is contraindicated. The term DHSt is preferred over hereditary xerocytosis because of the potential for confusion with hereditary spherocytosis (HS) because of their similarities in pronunciation, while splenectomy is indicated in HS but contraindicated in HX. In contrast, *KCNN4*-associated DHSt (sometimes referred to as *Gardos channelopathy*) have more severe anemia, lower reticulocyte count, and worse iron overload. EMA testing is also normal, but unlike *PIEZO1* mutations, ektacytometry profile is similar to normal patients in most cases. Therefore, targeted NGS can be helpful in identifying the causal mutation (see section on NGS). OHSt can be caused by mutations in either the *RHAG* (Rhesus-associated glycoprotein [RhAG], an ammonium transporter) or *SLC4A1* genes, in which the cation leak result in excess intracellular water content (overhydrated), with macrocytic anemia that varies from moderate to severe, and a low MCHC. As opposed to DHSt, OHSt produces a right shift on ektacytometry profile. Like other congenital hemolytic anemias, diagnosis of OHSt is based on a combination of clinical presentation, macrocytic anemia and reticulocytosis, familial history with an autosomal dominant inheritance pattern, and laboratory testing including targeted NGS. Management of both DHSt and OHSt is largely supportive and aims to reduce complications from anemia and iron overload.

Acanthocytosis

Spur cells, or acanthocytes (from the Greek *acantha*, or thorn; Figure 8-5), are erythrocytes with multiple irregular projections that vary in width, length, and surface

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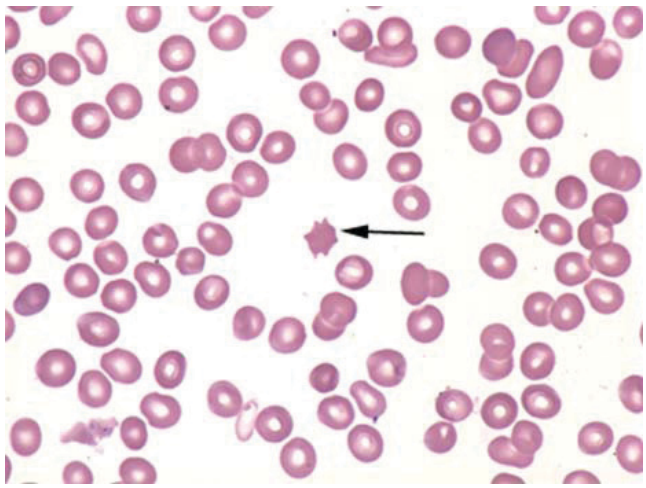

Figure 8-5 Acanthocytes.

distribution. Several conditions are associated with this morphology. In severe liver disease, acanthocyte formation is a 2-step process involving the transfer of free, nonesterified cholesterol from abnormal plasma lipoproteins into the erythrocyte membrane and then the subsequent remodeling of abnormally shaped erythrocytes by the spleen. Rapidly progressive hemolytic anemia is seen in association with advanced and often end-stage alcoholic cirrhosis, sometimes referred to as *Zieve syndrome*, or other conditions such as metastatic liver disease, cardiac cirrhosis, Wilson disease, and fulminant hepatitis.

Abetalipoproteinemia

In abetalipoproteinemia, the primary molecular defect involves a congenital absence of apolipoprotein B in plasma. Consequently, all plasma lipoproteins containing this apoprotein as well as plasma triglycerides are nearly absent. Plasma cholesterol and phospholipid levels also are markedly reduced. The role of these lipid abnormalities in producing acanthocytes is not well understood. The most striking abnormality of the acanthocyte membrane in abetalipoproteinemia is an increase in membrane sphingomyelin. Abetalipoproteinemia is an autosomal recessive disorder that manifests in the first month of life with steatorrhea. Retinitis pigmentosa and progressive neurologic abnormalities, such as ataxia and intention tremors, develop between 5 and 10 years old and progress to death by the second or third decade of life. Therefore, it is crucial that patients are diagnosed promptly upon suspicion of the disease so that treatment can be initiated to halt disease progression and recover normal neurological function. Treatment includes strict adherence to a low-fat diet, supplementation with essential fatty acids and fat-soluble vitamins. Patients also need to be monitored for ophthalmologic, neurologic, hematologic, and hepatic complications.

McLeod phenotype

Acanthocytes have also been described in patients with the McLeod phenotype, a condition in which the erythrocytes have reduced surface Kell antigen. The affected red cells lack the Kx protein, which is a membrane precursor of the Kell antigen and needed for its expression. The Kx antigenic protein is encoded by the X chromosome, so males are affected with mild compensated hemolysis and variable acanthocytosis (8% to 85%). Because of Lyonization, female carriers are asymptomatic with occasional acanthocytes, and may be identified by flow cytometric analysis of Kell blood group antigen expression. In some ethnicities, the frequency of the Kx antigen is >99%, and thus, individuals with the McLeod phenotype can develop major problems with alloimmunization after immunizing events such as a transfusion. As such, autologous donation should be considered where possible. The McLeod phenotype is a key feature of McLeod syndrome, a rare multisystem disease characterized by neuropsychiatric, neuromuscular, cardiac, and hematological abnormalities. The subtle hematological abnormalities may precede the neurological complications for decades until patients develop premature dementia, cognitive impairment, social retraction, personality changes, and a choreatic movement disorder or dystonia. McLeod phenotype has also been associated with X-linked granulomatous disease.

Rh deficiency (null) syndrome

This term is used to designate rare cases of either absent (Rh_{null}) or markedly reduced (Rh_{mod}) expression of the Rh antigen in association with mild-to-moderate hemolytic anemia. Three proteins (RhCE, RhD, and Rh50) compose the Rh protein family. This disorder arises through autosomal recessive inheritance of either a suppressor gene unrelated to the Rh locus or a silent allele at the locus itself. The normal, complexed structure forms an integral membrane protein; its loss disrupts membrane architecture. Rh_{null} cells have increased rates of cation transport and sodium-potassium membrane adenosine triphosphate (ATP)-ase activity that results in dehydrated RBCs. This dehydration results in stomatocytes and occasional spherocytes on the peripheral blood film. Laboratory evaluation shows increased RBC osmotic fragility, reflecting a marked reduction of the membrane surface area. The relationship between the absence of the Rh antigen proteins and RBC alterations leading to hemolysis

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presumably involves membrane microvesiculation, leading to diminished erythrocyte flexibility. Splenectomy results in improvement of the hemolytic anemia.

KEY POINTS

- HE/HPP is caused by defects in the interactions of red cell cytoskeleton proteins ("horizontal interactions"), with spectrin abnormalities accounting for most of the cases.
- The majority of patients with HE is not symptomatic and require no therapy.
- HPP is a severe form of HE where coinheritance of spectrin defects and an hypomorphic allele lead to a more severe phenotype with markedly abnormal red cells characterized by increased thermal instability.
- Splenectomy is contraindicated in patients with DHSt. Genetic testing to confirm the genotype of the membranopathy is advisable prior to splenectomy.

Abnormalities of RBC enzymes

CLINICAL CASE

A 23-year-old African American male who recently underwent cadaveric renal transplant for end-stage renal disease secondary to nephrotic syndrome is referred for urgent evaluation of anemia. When he left the hospital, his hemoglobin was 10.3 g/dL, with no evidence of graft rejection. His discharge medications included prednisone, cyclosporine, trimethoprim/sulfamethoxazole, and acyclovir. The day after discharge, he complains of acute onset of severe fatigue, dyspnea, and scleral icterus, but no fever or infectious symptoms. Physical examination revealed heart rate of 112, blood pressure of 89/45, and scleral icterus, but unremarkable otherwise. Hemoglobin is 6.6 g/dL, absolute reticulocyte count is 477×10^9/L, LDH 1543 U/L. Serum creatinine is 137 µmol/L and the platelet count 302×10^9/L, similar to hospital discharge. A moderate number of bite and blister cells and polychromatophilia are noted on the peripheral blood film.

Normal metabolism of the mature RBC involves 2 principal pathways of glucose catabolism: the glycolytic pathway and the hexose monophosphate shunt. The 3 major functions of the products of glucose catabolism in the erythrocyte are (1) maintenance of protein integrity, cellular deformability, and RBC shape; (2) preservation of hemoglobin iron in the ferrous form; and (3) modulation of the oxygen affinity of hemoglobin. These functions are served by the regulation of appropriate production of 5 specific molecules: ATP, reduced glutathione, reduced nicotinamide adenine dinucleotide (NADH), reduced nicotinamide adenine dinucleotide phosphate (NADPH), and 2,3-DPG. Maintenance of the biochemical and structural integrity of the RBC depends on the normal function of >20 enzymes involved in these pathways as well as the availability of 5 essential RBC substrates: glucose, glutathione, NAD, nicotinamide adenine dinucleotide phosphate, and adenosine diphosphate.

The primary function of the glycolytic pathway is the generation of ATP, which is necessary for the ATPase-linked sodium-potassium and calcium membrane pumps essential for cation homeostasis and the maintenance of erythrocyte deformability. The production of 2,3-DPG in this pathway is regulated by the Rapoport-Luebering shunt, which is controlled by bisphosphoglyceromutase, the enzyme that converts 1,3-DPG to 2,3-DPG. Concentration of 2,3-DPG in the RBC in turn regulates hemoglobin oxygen affinity, thus facilitating the transfer of oxygen from hemoglobin to tissue-binding sites. The major function of the hexose monophosphate shunt is preservation and regeneration of reduced glutathione via NADPH, which protects hemoglobin and other intracellular and membrane proteins from oxidant injury.

Abnormalities of the glycolytic pathway

Pyruvate kinase deficiency

PKD is the most common chronic nonspherocytic hemolytic anemia (CNSHA) caused by a defect in glycolytic RBC metabolism. PK enzymes are a product of 2 distinct genes, *PKLR* (encoding the liver [L] and RBC [R] isoenzymes) and *PKM* (encoding the muscle [M] isoenzyme). PKD is caused by mutations of the *PKLR* gene located in chromosome 1q21. Rarely, mutations in the Kruppel-like factor 1 (*KLF-1*) gene have also been shown to reduce PK activity. It is autosomal recessively inherited and clinically heterogeneous. The majority of the PKLR mutations are missense mutations, while others are stop codon or large deletions (non-missense mutations). PKD has a worldwide distribution with an estimated prevalence of 3 to 8 per 1,000,000; more than 300 pathogenic variants of the *PKLR* gene have been described.

Clinical manifestations

Presentation of PKD can range from neonatal jaundice (necessitating exchange transfusions in 46% of births), jaundice, splenomegaly, and failure to thrive in the neonatal period or early childhood, to a mild presentation with fully compensated hemolytic anemia. The broad range of presentation can be misleading (eg, hyperbilirubinemia and gallstones initially mistaken as Gilbert's syndrome later found to have compensated hemolysis), leading to delays in diagnosis and perhaps underdiagnosis. Patients with non-missense/non-missense mutations tend

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to be diagnosed earlier, with lower hemoglobin value, higher number of lifetime transfusions, and transfusion dependency compared to patients with non-missense/missense and missense/missense mutations. Because 2,3-DPG level is increased in the RBCs, anemia from PKD is better tolerated than anemias from other causes of hemolysis.

Laboratory evaluation

Peripheral blood films from patients with PKD may show small dense crenated cells (*echinocytes* or *prickle cells*). However, the RBC morphology is frequently normal. In the most severe cases, marked reticulocytosis, nucleated RBCs, and substantial anisopoikilocytosis can be seen. The MCV is usually normal or increased, reflecting the contribution of reticulocytes. A marked increase in the reticulocyte count (up to 70%) occurs after splenectomy in PKD. Reference laboratories can perform quantitative measurement of the erythrocyte enzyme activity necessary to diagnose this condition accurately. Reticulocytes have higher levels of PK and extreme reticulocytosis, commonly seen after splenectomy, may result in borderline low or even normal PK activity. In such a situation, it is essential to compare the activity of PK relative to the other RBC age-dependent enzymes such as glucose-6-phosphate dehydrogenase (G6PD) and hexokinase. A disproportionately "normal" PK level suggests PKD. Therefore, the diagnosis of PKD should always be supported by genetic testing of the *PKLR* gene.

Treatment and monitoring

Acute stressors, such as viral infections, may lead to increased hemolysis and worsening of the anemia, necessitating transfusions. Indications for regular transfusion include poor feeding or failure to thrive in children, symptomatic anemia, and its impact on quality of life. Splenectomy can be considered in those with frequent transfusions and symptomatic anemia but is not effective in all patients and only offers partial correction of the anemia. The decision to splenectomize need to be weigh against the risk of thrombosis and bacterial infection by encapsulated organisms. Patients will require presplenectomy and regular postsplenectomy vaccination per local guidelines. Patients should be monitored regularly for iron overload and its associated complications, even in the absence of transfusion, for gallstones and intrahepatic cholestasis after cholecystectomy with abdominal ultrasound, and bone density monitoring as up to 33% of PKD adults suffer from osteopenia and bone fractures. Pulmonary hypertension is rare (~5% of PKD adults) but, because of its associated morbidity and mortality, it should be surveyed in patients over 30 years of age, prior to pregnancy, or in the presence of cardiac symptoms. Clinical trials are ongoing to evaluate the efficacy and safety of small molecule PK activator and lentiviral vector gene therapy in PKD patients.

Other disorders of the glycolytic pathway

Both glucose phosphate isomerase and hexokinase deficiencies produce nonspherocytic hemolytic anemia associated with decreased erythrocyte ATP and 2,3-DPG content. These disorders are rare; patients often present in childhood with mild-to-moderate anemia and reduced exercise tolerance. A form of acquired hexokinase deficiency occurs in Wilson disease, in which elevated copper levels in the blood inhibit hexokinase in a fluctuating fashion that may lead to intermittent brisk intravascular hemolysis. Phosphofructokinase deficiency was first described as a muscle glycogen storage disease; some patients with this deficiency have a chronic hemolytic anemia. In phosphofructokinase deficiency, low levels of erythrocyte ATP lead to low-grade hemolysis, but the limiting symptoms are usually weakness and muscle pain on exertion. Children with phosphoglycerate kinase have associated neuromuscular manifestations, including seizures, spasticity, and mental retardation.

Abnormalities of the hexose monophosphate shunt

G6PD is the major enzyme involved, because deficiencies of the 2 downstream enzymes 6-phosphogluconolactonase and 6-phosphogluconate dehydrogenase are extremely rare and not always associated with hemolysis.

G6PD deficiency

G6PD deficiency is the most frequently encountered abnormality of RBC metabolism, affecting >500 million people worldwide. The gene for G6PD is carried on the X chromosome and exhibits extensive polymorphism. Enzyme deficiency is observed in males carrying a variant gene. Females manifesting G6PD deficiency may be homozygous, compound heterozygous, or heterozygous with unfavorable Lyonization (skewed inactivation of the X chromosome bearing the wild-type G6PD allele). Worldwide, 230 genetic variants of G6PD have been described and are categorized by the World Health Organization according to the extent of enzyme deficiency and severity of hemolysis: class I (CNSHA), class II (<10% activity and intermittent hemolysis), class III (10% to 60% activity and intermittent hemolysis), class IV (normal activity and no hemolysis), and class V (increased activity).

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G6PD B, the wild-type enzyme, and G6PD A^+, a common variant in the African American population, demonstrate normal enzyme activity and are not associated with hemolysis. G6PD A^- is present in approximately 10% to 15% of African American males. This variant is an unstable enzyme, with a decrease in enzyme activity in aged RBCs. Hemolysis is typically self-limited. In contrast, other G6PD variants have reduced catalytic activity and marked instability, rendering both reticulocytes and older cells susceptible to hemolysis. Enzymatic deficiency of this type is seen in up to 5% of persons of Mediterranean or Asian ancestry, as well as Ashkenazi Jews. The common example of this deficiency is G6PD Mediterranean.

Pathophysiology

Hemolysis in G6PD-deficient RBCs is caused by a failure to generate adequate NADPH, a reducing agent required in the conversion of hydrogen peroxide to water by all 3 detoxification pathways in an RBC: the glutathione, thioredoxin/peroxiredoxin-2, and catalase pathways. In steady state, even most G6PD-deficient variants are able to cope with the endogenous auto-oxidation of 2% to 3% of total hemoglobin per day. Under increased oxidative challenge from exogenous sources like certain drugs and infection, these red cells are no longer able to keep up with the demand for NADPH, rendering the red cell susceptible to oxidation of hemoglobin and cellular proteins by reactive oxygen species. The resulting denatured hemoglobin aggregates and forms intraerythrocytic Heinz bodies, which bind to membrane cytoskeletal proteins, and together with denatured membrane proteins leading to decreased cellular deformability. Cells containing Heinz bodies are entrapped or partially destroyed in the spleen, resulting in loss of cell membranes through pitting of Heinz bodies and leading to hemolysis. Of note, Heinz bodies are not specific to G6PD and can be seen in thalassemias and unstable hemoglobinopathies. If the oxidant stress is severe, intravascular hemolysis may occur.

The severity of hemolytic anemia in patients with G6PD deficiency depends on the type of defect, the level of enzyme activity in the erythrocytes, and the severity of the oxidant challenge. Ingestion of an oxidant drug or fava beans is sometimes the precipitating cause (Table 8-3). Some drugs have been confirmed to cause hemolysis in G6PD-deficient individuals, while for others no firm evidence exists to implicate their association and disagreements exist between literature sources. Hemolytic anemia in patients with G6PD deficiency may first be recognized during an acute clinical event that induces oxidant stress, such as infection, diabetic ketoacidosis, or severe liver injury. In children, infection is a common precipitating event. Fava beans can trigger severe hemolysis, a condition termed *favism*, caused by divicine and isouramil in the beans. Patients may not recognize that they have ingested fava beans as they go by other names, such as *broad beans*, in different parts of the world.

Clinical manifestations, laboratory evaluation, and treatment

The most common presentation is acute hemolysis provoked by oxidant drug or illness. Favism is less common except in Southern Europe, the Middle East, and Southeast Asia, where fava beans are a popular food. However,

Table 8-3 Drugs that can cause clinically significant hemolysis in patients with G6PD deficiency

Drug category	Predictable hemolysis	Possible hemolysis	Possible association (less likely)
Antimalarials	Dapsone-containing combinations Primaquine Pamaquine Tafenoquine	Chloroquine Quinidine Quinine	
Analgesics/antipyretics	Phenazopyridine (Pyridium)	Aspirin (high dose)	
Antibacterials	Trimethoprim-sulfamethoxazole Sulfadiazine Quinolones (nalidixic acid, ciprofloxacin, ofloxacin, moxifloxacin) Nitrofurantoin	Sulfasalazine Sulfadiazine	Chloramphenicol Isoniazid
Other	Henna Methylene blue Niridazole Pegloticase Rasburicase	Menadiol sodium phosphate Toluidine blue (Tolonium chloride)	Ascorbic acid Dimercaptosuccinic acid Glyburide (glibenclamide) Isosorbide dinitrate Mepacrine Vitamin K analogs

Adapted from Luzzatto L et al, *Blood*. 2020;136(11):1225-1240 and Youngster I et al, *Drug Safety*. 2010;33(9):713-726.

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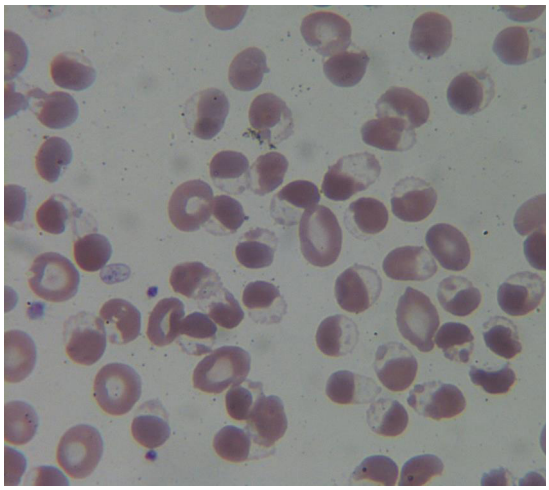
hemolytic anemia caused by favism may be severe or even fatal, particularly in children. G6PD deficiency predisposes to neonatal jaundice, and it may be the result of impairment of hepatic function, hemolysis, or both. Certain rare G6PD variants may result in CNSHA with severe neonatal jaundice, persistent splenomegaly and jaundice, gallstones, and sometimes transfusion dependency.

G6PD deficiency should be considered in an individual with evidence of chronic DAT-negative hemolysis or infants with neonatal jaundice, which usually peaks on days 2 to 3 after birth. Paradoxically, unlike other causes of neonatal jaundice, hemolysis is mild to absent in patients with G6PD deficiency. The peripheral blood film may show RBCs with the hemoglobin confined to one side of the cells, with the remainder appearing as a hemoglobin-free ghost (eccentrocytes) (Figure 8-6). The morphology has previously been described as *bite* or *blister cells*, interpreted as the result of removal of denatured hemoglobin by the spleen; however, it appears that the accumulated oxidized hemoglobin actually remains and is adherent to the RBC membrane. Brilliant cresyl blue staining may reveal Heinz bodies. Screening or quantitative biochemical assays can be used to make the diagnosis. In less severe G6PD variants, during an acute hemolytic episode, an elevated reticulocyte count raises the mean level of erythrocyte G6PD and render a false-negative result. G6PD levels, therefore, should be checked several months after the acute event when there will be RBCs of varying ages. As previously discussed in the section on PKD, comparative assessment of the activity levels of unaffected RBC enzymes (eg, hexokinase or pyruvate kinase) may help in the test interpretation during an acute episode.

Figure 8-6 G6PD deficiency. The peripheral blood film shows several red cells with the hemoglobin confined to one side of the cells, with the remainder appearing as a hemoglobin-free ghost (eccentrocytes). Source: ASH Image Bank/Dipen C. Patel, Marc Matrana, and Courtney Jackson (image 00061028).



Management is primarily avoidance or discontinuation of oxidant drugs and avoidance of fava beans. Patients with neonatal jaundice will require phototherapy, and in some cases exchange transfusion, to avoid kernicterus. When anemia is severe, RBC transfusion may be necessary. Patients with CNSHA who are transfusion-dependent may benefit from splenectomy.

Abnormalities of nucleotide metabolism

Pyrimidine-5′-nucleotidase (P5′N-1) deficiency is an enzymatic abnormality of pyrimidine metabolism associated with CNSHA. Like other congenital hemolytic anemias, patients usually have lifelong hemolysis, mild-to-moderate anemia, jaundice, splenomegaly, gallstones, and iron overload. Neonatal jaundice has been observed in some patients. The peripheral blood film made from fresh blood sample in patients with this defect often shows polychromatic macrocytic RBCs containing fine to coarse basophilic stippling, from the precipitation of accumulated excess pyrimidine nucleotide. Lead intoxication also inactivates the enzyme, leading to an acquired variant of pyrimidine-5′-nucleotidase deficiency.

Adenosine deaminase excess is an unusual abnormality and is the only RBC enzyme disorder that is inherited in an autosomal dominant manner, but the molecular mechanism of this disorder has not been identified. It is caused by a genetically determined increase in the activity of a normal erythrocyte enzyme. The excessive deaminase activity prevents normal salvage of adenosine and causes subsequent depletion of ATP and hemolysis. Deficiency of adenosine deaminase is associated with severe combined immunodeficiency.

CLINICAL CASE *(continued)*

The patient presented in this section should be suspected of having G6PD deficiency. Patients with the African American variant (G6PD A$^-$) are often asymptomatic until they ingest medication or experience an infection, which leads to oxidant stress of the RBCs. Trimethoprim/sulfamethoxazole was likely the offending agent. During the early phases of hemolysis, eccentrocytes can be seen on peripheral blood film. A Heinz body preparation may show the typical inclusions, which consist of denatured hemoglobin. G6PD levels may be misleading in the acute setting, as values may be normal because of reticulocytosis. Treatment is primarily supportive. Offending drugs should be discontinued and alternative agents chosen. If the prescribed agent cannot be substituted, a trial of continuation is reasonable, as hemolysis often is compensated in the G6PD A$^-$ variant.

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Targeted next-generation sequencing

As indicated previously, intrinsic abnormalities of the RBC are phenotypically diverse and genetically heterogeneous. Extensive biochemical workup may be inconclusive and multiple rounds of single-gene testing are costly to perform. Various NGS panels have been independently developed in the past decade that allow high-throughput sequencing of multiple candidate genes in parallel. Targeted NGS is the approach most often chosen in determining the molecular basis of congenital hemolytic disorders. Targeted NGS involves sequencing genes known to cause congenital hemolytic disorders and is especially helpful in severe cases that are heavily transfused and therefore functional biochemical studies cannot be performed, or in cases where the amount of blood required for biochemical testing is too large (eg, enzyme assays in neonates). Genes chosen vary from panel to panel, varying from 50 to 200 genes. The average number of times the coding region, splice site junctions, intronic and regulatory regions are sequenced to improve accuracy (known as coverage) also varies between panels (a minimum of 50 times or >90% of target regions should be covered at least 20 times in diagnostic settings). Costs, accuracy, time, and read length vary between NGS platforms. As such, the diagnostic yield of a targeted NGS approach can vary between 38% and 87%, depending on how many and what types of genes are included and the depth of the phenotypic assessment; the results should be interpreted in light of these factors. In these cases, there may be a need to proceed to whole-exome sequencing or genome sequencing to elucidate the existence of a new causal variant or new causal genes. Even then, whole-exome sequencing and whole-genome sequencing are not overly sensitive methods to detect long insertion-deletion, copy-number, structural, and epigenetic variants. Therefore, targeted NGS is not useful for ruling out a genetic cause in congenital hemolytic disorder. The clinical significance of isolated variants should be established via clinical history, physical examination, laboratory tests, and family history, along with previous reports of known pathogenicity, to determine penetrance and mode of inheritance. Sometimes further laboratory testing and experimentation are required to determine the pathogenicity of the variant in question. This is especially true in cases where the clinical presentation is atypical. Such cases are usually referred to research or reference centers for further investigations. The American College of Medical Genetics and Genomics has established a guideline for variant interpretation.

KEY POINTS

- The glycolytic pathway generates ATP, which is necessary for maintenance of RBC membrane integrity and oxygen affinity.
- Glucose metabolism through the hexose monophosphate shunt produces NADPH to maintain the antioxidative activity of the RBC.
- Enzymopathies represent a major consideration in the differential diagnosis of inherited DAT-negative nonspherocytic hemolytic anemias.
- PKD is the most common defect of the glycolytic pathway and G6PD deficiency and the most common defect of the hexose monophosphate shunt.
- In G6PD deficiency, quantitative measurement of the enzyme levels during an acute hemolytic episode may be falsely elevated.
- Defects of purine and pyrimidine metabolism are infrequent. The peripheral blood film in pyrimidine-5′-nucleotidase deficiency shows red cells with coarse basophilic stippling.
- Iron overload can occur in nontransfused congenital hemolytic anemias.
- Targeted NGS may be helpful in determining the causal variants responsible for the congenital hemolytic anemia.

Hemolysis caused by extrinsic abnormalities of the RBC

CLINICAL CASE

A 42-year-old African American woman presents to the emergency room with malaise and progressive dyspnea over 3 days. She has never been pregnant or had a prior blood transfusion. She has no other medical history. Testing reveals severe anemia, marked hemolysis, mild thrombocytopenia, and DAT test was positive. Blood bank testing was interpreted as "no units compatible for transfusion." Labs reveal: Hgb 3.7 g/dL, platelets 60 × 109/L, LDH 1450 U/L, absolute reticulocyte count of 7 × 109/L, MCV 118.7 fL, haptoglobin <6mg/dL , total bilirubin 6.6 mg/dL, urine positive for hemoglobin pigment but negative for RBC. A DAT is positive for immunoglobulin G (IgG) (panagglutinin), and negative for C3. Smear shows microspherocytes. Hours later she is found with respiratory distress with chest pain and confusion.

Hemolytic anemia caused by immune injury to RBCs

Autoimmune hemolytic anemia (AIHA) is a family of immune-mediated disorders precipitated by autoantibodies against shared RBC antigens. Most cases of AIHA in adults are mediated by IgG that recognizes RBC at

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physiologic temperature (37°C), termed *warm AIHA*. Immunoglobulin M (IgM) autoantibodies are usually clinically relevant at lower temperatures, termed *cold AIHA*. IgG or IgM can activate complement, which leads to C3 deposition on the RBC as detected in the DAT. Some patients can have both IgG and IgM, termed *mixed AIHA*, and around 10% fail to demonstrate a positive Coombs test, which is termed *atypical* or *Coombs-negative AIHA*.

The classifications are further characterized as *primary* or *secondary*. In primary AIHA no clear provoking or associated condition is present (ie, lupus, lymphoma, infection, immunodeficiency). Secondary AIHA occurs when the AIHA is associated with an underlying disorder; however, this disorder may not be evident on presentation. Evaluation for secondary causes is helpful as this may change medical management. The classifications of AIHA are shown in Table 8-4.

These naming conventions are cumbersome, and hematologists must be mindful of assay limitations. For example, a patient with IgM-mediated AIHA can have an antibody causing hemolysis at 37C. Indeed, "warm IgM AIHA" is quite nefarious. The patient with C3+ but IgM and IgG negative AIHA may prompt an extensive evaluation for oddities, such as paroxysmal cold hemoglobinuria (PCH), but this result can be observed with a low-affinity IgG, low-abundance IgG, or non-IgG antibody. Atypical or Coombs-negative AIHA merits special discussion. The number of IgG molecules necessary to cause agglutination in a Coombs test can vary with reagents and test methodology (conventional tube versus gel tube); in addition, patients may have pathological IgG bound to RBCs below the detection limit of the DAT assay used. Similar subthreshold complement deposition in patients with hemolysis but negative DAT has also been observed.

Table 8-4 Classifications of immune injury to red blood cells

I. Warm-autoantibody type: autoantibody maximally active at 37°C
A. Primary or idiopathic warm AIHA
B. Secondary warm AIHA
1. Associated with lymphoproliferative disorders (eg, chronic lymphocytic leukemia, non-Hodgkin lymphoma)
2. Associated with the rheumatic disorders (eg, SLE)
3. Associated with certain nonlymphoid neoplasms (eg, ovarian tumors)
4. Associated with certain chronic inflammatory diseases (eg, ulcerative colitis)
5. Associated with certain drugs (eg, cephalosporins, NSAIDs)
II. Cold-autoantibody type: autoantibody optimally active at temperatures <37°C
A. Mediated by cold agglutinins
1. Idiopathic (primary) chronic cold agglutinin disease (usually associated with IgMκ monoclonal gammopathy of undetermined significance)
2. Secondary cold agglutinin hemolytic anemia
a. Postinfectious (eg, *Mycoplasma pneumoniae* or infectious mononucleosis)
b. Associated with malignant B-cell lymphoproliferative disorder
B. Mediated by cold hemolysins
1. Idiopathic (primary) paroxysmal cold hemoglobinuria
2. Secondary paroxysmal cold hemoglobinuria
a. Associated with an acute viral syndrome in children
b. Associated with congenital or tertiary syphilis in adults
III. Mixed cold and warm autoantibodies
A. Primary or idiopathic mixed AIHA
B. Secondary mixed AIHA
1. Associated with the rheumatic disorders, particularly SLE
IV. Drug-immune hemolytic anemia
A. Hapten or drug adsorption mechanism
B. Ternary (immune) complex mechanism
C. True autoantibody mechanism

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NSAID, nonsteroidal anti-inflammatory drug; SLE, systemic lupus erythematosus.

Pathophysiology

Warm AIHA

The most common type of AIHA is warm-reactive IgG, but non-IgG forms can occur. The causative IgG antibodies are polyclonal and target shared RBC structural antigens, such as glycophorin A, band 3, and the RH system. Since the autoantibodies bind to RBC regardless of blood type determinants (Kell, Duffy, etc.), they are referred to as a *panagglutinin*. The IgG predominantly leads to splenic macrophage destruction of the RBC but can also activate terminal complement. It is important to note that these autoantibodies are distinct from alloantibodies. Alloantibodies develop after exposure to a nonself antigen, such as when a Kell-negative patient receives Kell-positive blood. Autoantibodies bind to a patient's own RBCs, and as a result the antibodies are expected to bind to donor cells. Not all antibodies are pathological. Patients who are healthy, pregnant, or who have autoimmune diseases may have positive testing without evidence of hemolysis; silent antibodies do not signify that the patient will develop of AIHA at later times.

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Cold AIHA

Cold-reactive antibodies are usually IgM and bind to RBCs at temperatures less than 37°C. The IgM pentamer structure allows direct agglutination of RBCs. The IgM can potently activate complement, which causes C3 deposition on the red cells. C3 deposition can mediate RBC engulfment by splenic or hepatic macrophages, and cause RBC lysis from terminal complement. *Cold agglutinin syndrome* refers to IgM AIHA as a transient phenomenon, often associated with infections such as *Mycoplasma* or Epstein-Barr virus. *Cold agglutinin disease* refers to IgM AIHA from a recently characterized chronic lymphoproliferative disorder. In cold AIHA, the IgM is reactive against I or i antigen.

Cold reacting IgG antibody (Donath-Landsteiner), against P antigen is seen in PCH. In PCH, the IgG binds to RBC in the cooler peripheral circulation, but complement-mediated destruction occurs when the RBC warms. This is a rare etiology in modern medicine.

Drug-induced immune hemolytic anemia

A large number of drugs can cause hemolytic anemia; careful medication review and history are needed to discern them (Table 8-5). Three basic mechanisms of drug-induced immune RBC injury are recognized. A fourth mechanism may lead to nonimmunologic deposition of multiple serum proteins, including immunoglobulins, albumin, fibrinogen, and others on the RBC, but RBC injury does not occur. The mechanisms of drug-induced immune hemolytic anemia and positive DATs are summarized in Table 8-6. In recent series, cephalosporins, penicillin derivatives, and nonsteroidal anti-inflammatory drugs account for >80% of drug-induced immune hemolytic anemia.

Hapten or drug adsorption mechanism

Hapten or drug adsorption mechanism applies to drugs that bind firmly to proteins on the RBC membrane. The classic setting is very high-dose penicillin therapy, but other drugs such as cephalosporins and semisynthetic penicillins also are implicated. The antibody responsible for hemolytic anemia by this mechanism is of the IgG class and is directed against epitopes of the drug. Other manifestations of drug sensitivity, such as hives or anaphylaxis, usually are not present. The antibody binds to drug molecules attached to the RBC membrane. Antibodies eluted from patients' RBCs or present in their sera react in the indirect antiglobulin test only against drug-coated RBCs, which distinguishes these drug-dependent antibodies from true autoantibodies. Destruction of RBCs coated with drug and IgG antidrug antibody occurs mainly through sequestration by splenic macrophages. Hemolytic anemia typically occurs 7 to 10

Table 8-5 Drugs associated with immune injury to red blood cells or a positive direct antiglobulin test

Hapten or drug adsorption mechanism	
Carbromal	Oxaliplatin
Cephalosporins	Penicillins
Cianidanol	Tetracycline
Hydrocortisone	Tolbutamide
6-Mercaptopurine	
Ternary immune complex mechanism	
Amphotericin B	Nomifensine
Antazoline	Oxaliplatin
Cephalosporins	Pemetrexed
Chlorpropamide	Probenecid
Diclofenac	Quinine
Diethylstilbestrol	Quinidine
Doxepin	Rifampicin
Etodolac	Stibophen
Hydrocortisone	Thiopental
Metformin	Tolmetin
Autoantibody mechanism	
Cephalosporins	Lenalidomide
Cianidanol	Mefenamic acid
Cladribine	α-Methyldopa
Diclofenac	Nomifensine
L-DOPA (levodopa)	Oxaliplatin
Efalizumab	Pentostatin
Fludarabine	Procainamide
Glafenine	Teniposide
Latamoxef	Tolmetin
Nonimmunologic protein adsorption	
Carboplatin	Cisplatin
Cephalosporins	Oxaliplatin
Uncertain mechanism of immune injury	
Acetaminophen	Melphalan
p-Aminosalicylic acid	Mephenytoin
Carboplatin	Nalidixic acid
Chlorpromazine	Omeprazole
Efavirenz	Phenacetin
Erythromycin	Streptomycin
Fluorouracil	Sulindac
Ibuprofen	Temafloxacin
Insecticides	Thiazides
Isoniazid	Triamterene

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Table 8-6 Immune hemolytic anemia and positive direct antiglobulin reactions caused by drugs

	Hapten-drug adsorption	Ternary immune complex formation	Autoantibody formation	Nonimmunologic protein adsorption
Prototype drug	Penicillin	Third-generation cephalosporins	α-Methyldopa	Cephalothin
Role of drug	Binds to red cell membrane	Forms 3-way complex with antibody and red cell membrane component	Induces antibody to native red cell antigen	Possibly alters red cell membrane
Drug affinity to cell	Strong	Weak	None demonstrated	Strong
Antibody to drug	Present	Present	Absent	Absent
Antibody class predominating	IgG	IgM or IgG	IgG	None
Proteins detected by direct antiglobulin test	IgG, rarely complement	Complement	IgG, rarely complement	Multiple plasma proteins
Dose of drug associated with positive antiglobulin test	High	Low	High	High
Mechanism of red cell destruction	Splenic sequestration	Direct lysis by complement plus splenic sequestration	Splenic sequestration	None

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days after the drug is started and ceases a few days to 2 weeks after the patient discontinues taking the drug.

Ternary or immune complex mechanism: drug antibody–target cell interaction

Drugs in this group exhibit only weak direct binding to blood cell membranes. A relatively small dose of drug is capable of triggering destruction of blood cells. Blood cell injury is mediated by a cooperative interaction among 3 reactants to generate a ternary complex:1) the drug or a drug metabolite, 2) drug-binding membrane site (an antigen) on the target cell, and 3) a drug-dependent antibody. The pathogenic antibody recognizes the drug only in combination with a particular membrane structure of the RBC (eg, a known alloantigen). Binding of the drug to the target cell membrane is weak until the attachment of the antibody to *both* drug and cell membrane is stabilized. Yet the binding of the antibody is drug-dependent. RBC destruction occurs intravascularly after completion of the whole complement sequence, often resulting in hemoglobinemia and hemoglobinuria. The DAT is positive usually only for complement.

Autoantibody mechanism

Several drugs, by unknown mechanisms, induce the formation of autoantibodies reactive with RBCs in the absence of the instigating drug. The most studied drug in this category has been α-methyldopa, but levodopa and other drugs have also been implicated. Patients with chronic lymphocytic leukemia treated with pentostatin, fludarabine, or cladribine may have severe and sometimes fatal autoimmune hemolysis, although the mechanisms of autoantibody induction are likely different, most likely involving dysregulation of T lymphocytes. Patients treated with the anti-CD38 agent daratumumab will have a positive DAT suggestive of a panagglutinin because CD38 is present on RBC; intravenous immunoglobulin (IVIG) administration can also result in a positive DAT.

Nonimmunologic protein adsorption

A small proportion (<5%) of patients receiving cephalosporin antibiotics, carboplatin, and cisplatin develop a positive DAT because of nonspecific adsorption of plasma proteins to their RBC membranes. This process may occur within 1 to 2 days after the drug is instituted. Multiple plasma proteins, including immunoglobulins, complement, albumin, fibrinogen, and others, may be detected on RBC membranes in such cases. Hemolytic anemia resulting from this mechanism does not occur. This phenomenon, however, may complicate crossmatch procedures unless the drug history is considered.

Clinical manifestations and laboratory findings

Several clinical features of AIHA are common to all disease types. Patients may present with signs and symptoms of anemia (eg, dyspnea, weakness, dizziness), jaundice, abdominal pain, and fever. Mild splenomegaly is common. Hepatomegaly and lymphadenopathy may be evident at presentation depending on the etiology. Anemia may vary from mild to severe/life-threatening, usually with either

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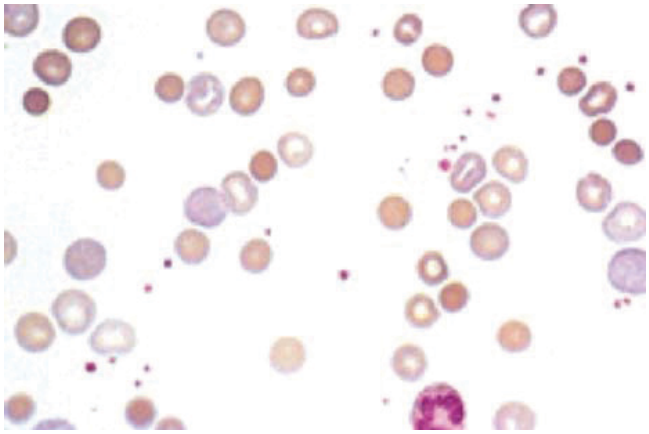
normocytic or macrocytic cells. Patients most frequently present with reticulocytosis. Reticulocytopenia, however, is especially worrisome because the uncompensated anemia can rapidly become life-threatening. Indirect bilirubin and LDH are elevated to varying degrees, and the haptoglobin is decreased, often below the limit of detection. The blood smear often demonstrates microspherocytes (Figure 8-7). Nucleated RBCs also may be present. In secondary cases, the presenting complaint may be related to the underlying disease, ie, joint pain in lupus, however AIHA may be the presenting manifestation of previously undiagnosed diseases such as lymphoma or immunodeficiency.

Patients with Cold Agglutinin Disease (CAD) usually have mild-to-moderate chronic hemolysis, punctuated by acute exacerbations that may be provoked by cold exposure, including a cold operating room during surgery. Symptoms such as acrocyanosis or Raynaud's are common. Spontaneous autoagglutination of RBCs at room temperature may be seen as clumps of cells on the blood smear (Figure 8-8). Laboratory evaluation will require quickly transporting blood samples to the lab with hot packs, to prevent agglutination after phlebotomy which can affect testing results. Abnormalities in cell counts, MCV, MCHC, and chemistries can occur from agglutination of cells before cell counting or from hemolysis.

Drug-immune hemolytic anemia caused by the hapten or true autoantibody mechanism is usually mild. In contrast, hemolysis caused by the ternary or immune complex mechanism can be acute in onset, severe, and sometimes fatal.

Understanding the methods used in the DAT are necessary to correctly interpret results. Discussion with the blood bank or transfusion medicine can be invaluable and

Figure 8-7 Warm antibody autoimmune hemolytic anemia. Note the small round spherocytes and the large, gray polychromatophilic erythrocytes.



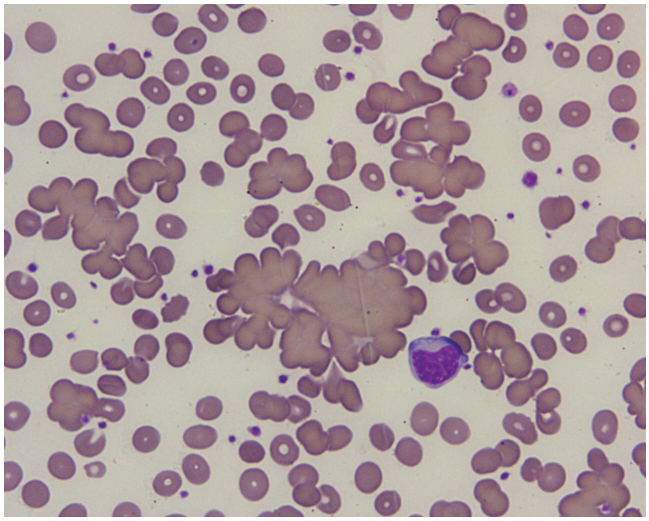


Figure 8-8 Cold agglutinin disease. Source: ASH Image Bank/John Lazarchick (image 00001053).

can avert delays in patient care because of miscommunication or uncertainty. Conventional tube tests assess RBC agglutination in small test tubes and the degree of agglutination is then graded visually. Gel systems ("gel cards" or "gel tubes") use a polymer to separate agglutinated RBC with centrifugation and agglutination is then graded visually. Both methods use polyclonal reagents against antibody and complement to induce agglutination, but the recognized components (IgG versus IgG and Immunoglobulin A [IgA]) depend on the manufacturer. If patient RBCs harbor either IgG or complement, the polyclonal reagent will cause agglutination. Further testing after a positive polyclonal agglutination with monoclonal reagents determines if IgG and C3 are each present individually. Once an antibody is identified, it can be eluted from the patient RBC and its specificity determined by analysis against RBC panels of known blood antigens. For example, in warm IgG AIHA, the eluted antibody will react with all RBC panels because it binds to shared structural antigens and is thus panagglutinin or may be described as "antibody of no apparent specificity." The major reaction patterns of the DAT and their differential diagnosis are summarized in Table 8-7.

The DAT by either tube or gel methods has limitations. Temperature-dependent or low-affinity antibodies may escape discovery: reagents may not detect IgA antibodies, antibodies may be removed from cells by washing, IgG or C3 fragments may be below the threshold of detection. Coombs-negative or atypical AIHA may occur from low-abundance IgG (<100 to 400 IgG/RBC), that is below the threshold of some assays (200 to 500 Ig/RBC). In addition, patients may have a positive DAT with

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Table 8-7 Differential diagnosis of reaction patterns of the direct antiglobulin test

Reaction pattern	Differential diagnosis
IgG alone	Warm antibody autoimmune hemolytic anemia
	Drug-immune hemolytic anemia: hapten/drug adsorption type or autoantibody type
Complement alone	Warm antibody autoimmune hemolytic anemia with subthreshold IgG deposition
	Cold agglutinin disease
	Paroxysmal cold hemoglobinuria
	Drug-immune hemolytic anemia: ternary immune complex type
IgG plus complement	Warm antibody autoimmune hemolytic anemia
	Drug-immune hemolytic anemia: autoantibody type (rare)

no signs of hemolysis. Positive DAT can occur in 1:10,000 blood donors without disease, after IVIG therapy, and with modern therapies such as daratumumab that recognize an antigen present on RBC (CD38). In DAT-negative cases of hemolysis, tests such as "Super Coombs" or concentrated eluates may identify antibodies. The strength of the DAT (1+ versus 3+) has poor correlation with hemolysis; indeed, the cells being analyzed are the cells that have survived autoimmune attack.

Additional tests in CAD include antibody titer and thermal amplitude. Patients with CAD typically have a titer of 1:64 or greater. The titer means the number of dilutions of serum needed to cause agglutination of cells. 1:1024 is a more potent agglutinating antibody than 1:4. Thermal amplitude testing determines agglutination based on temperature, some antibodies may cause agglutination at temperatures not routinely encountered, whereas others will bind at temperatures encountered in the normal peripheral circulation. In this fashion, a cold agglutinin active at 4°C may not be relevant, however a cold agglutinin active at 34°C is expected to cause hemolysis and symptoms at temperatures found in the peripheral circulation.

Treatment

Patients with severe anemia and symptoms may require emergent blood transfusion and AIHA-directed therapy. Fatal AIHA is not rare. Those with mild compensated anemia may have clinical worsening, necessitating therapy at some point in the future. Severe anemia from AIHA is a medical emergency akin to major hemorrhage, but the immediate threat to life may not be appreciated. In animal models of severe anemia, sudden decompensation can occur, and in human studies high mortality is correlated with the degree of acute anemia. The IgG panagglutinin can cause delays in lifesaving transfusion because it will react with all donor units, hence an "incompatible" crossmatch. This is expected from the biology of AIHA. There is often an unrealistic fear that the panagglutinin can mask an underlying alloantibody, and that blood transfusion will worsen the problem, but transfusion of emergency-release ABO-matched blood may be required to avoid cardiovascular collapse. ABO-matched blood will be hemolyzed similar to the patient's endogenous RBCs, but ABO compatibility reactions can be avoided with ABO matching. Important patient history includes prior blood transfusions, pregnancies, and/or miscarriages—if these are absent, alloimmunization is not expected and transfusion should not be delayed. The phrase "least incompatible blood" is best avoided; all units are expected to be serologically incompatible and a quixotic search to identify "least incompatible blood" can cause delays, leading to adverse events. Patients can still hemolyze despite receiving "least incompatible" units. Reticulocytopenia, respiratory distress, chest pains, or altered mental status in a patient with severe anemia from AIHA are ominous findings. Involvement of transfusion medicine or the blood bank by the treating clinicians may avert delays and prevent fatal outcomes.

Warm AIHA

Corticosteroids are a first-line therapy. Treatment with prednisone 1 mg/kg daily (with capped doses for obese individuals between 60 and 100mg) is effective. However both corticosteroid toxicity and AIHA relapse are common, and long-term control will often require other agents. Randomized trials have shown the superiority of rituximab with corticosteroids over corticosteroid monotherapy, with responses of 75% with rituximab versus 31% to 36% with prednisone monotherapy at 1 year. Of note, one study found a significant survival advantage with rituximab because of fewer infection-associated deaths in the rituximab arm. Once disease remission is attained, steroids can be tapered to minimize steroid toxicity (mood changes, weight gain, sleep disturbances, GI upset). Usually decreases of 10 to 20mg weekly are well-tolerated. Once a daily dose of prednisone 20 mg is reached, slower tapers of 5 to 10 mg weekly are advised. If patients have relapsed disease, retreatment with rituximab can be considered. Use of other agents is well-described in the literature; however, there are no United States Food and Drug Administration (FDA)-approved therapies for AIHA, which can pose problems for attaining insurance approval. Many effective AIHA therapies are prohibitively expensive without insurance

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coverage. Mycophenolate mofetil, azathioprine, cyclosporine, cyclophosphamide (oral, IV, and high-dose IV), danazol, and bortezomib have been described for refractory AIHA. In some acute cases the use of IVIG (response rare 39.7%) or erythropoietin (case reports, abstracts) may be beneficial, but data are more limited. Patient preferences and medication side effect profiles can help select therapies in the relapsed/refractory setting.

Splenectomy is an effective therapy (response rates 70% to 80%) and may be reasonable if medical therapy is not effective. However, postsplenectomy sepsis is a devastating complication and can occur years or decades after the procedure; postsplenectomy sepsis had a reported incidence of 3.2%, with 1.4% mortality. If splenectomy is used, immunizations against pneumococcus, *H. influenzae*, and meningococcus are recommended.

Cold agglutinin disease

Most patients with CAD have symptoms precipitated by cold, and cold avoidance can improve hemolysis and symptoms. Corticosteroids are not effective in CAD, given the toxicity of the high doses needed for the temporary responses seen. Rituximab is a standard therapy for CAD, with response rates of about 50%. Other agents include bendamustine, bortezomib, and ibrutinib. Splenectomy is usually not helpful, as C3b-labeled cells are cleared by hepatic macrophages. In severe or refractory cases, plasmapheresis and eculizumab have been used. Since CAD hemolysis is mediated by the classical pathway of complement, complement inhibition has been explored for therapy. Although the terminal complement inhibitor eculizumab can decrease RBC lysis from terminal complement, C3b-labeled RBCs are still destroyed in the liver; hence, hemoglobin increases by less than 1g/dL in those treated. Sutimlimab, a C1s inhibitor, was shown to improve anemia (by 3.9 g/dL) and reduce hemolysis in patients with CAD but did not change acrocyanosis symptoms consistent with its mechanism of action.

CLINICAL CASE *(continued)*

This patient had a severe presentation of warm IgG AIHA and had Evans syndrome, concomitant AIHA, and immune thrombocytopenia. She experienced delay in transfusion because of the serologic incompatibility caused by AIHA and became critically ill from severe anemia. She was successfully treated with emergency-released ABO-matched blood transfusion without a reaction. She required treatment with IVIG, corticosteroids, rituximab, and mycophenolate. Misinterpreting crossmatch compatibility or searching for "least incompatible blood" in AIHA can lead to delays in therapy needed to prevent cardiovascular collapse.

KEY POINTS

- Warm-antibody–induced immune hemolytic anemia is typically IgG-mediated and results in spherocytic red cells.
- CAD is IgM-mediated with associated complement activation. The peripheral blood film reveals RBC agglutination and spherocytes.
- A variety of drugs cause immune hemolytic anemia. Clinical laboratory support of the diagnosis may not be available. Discontinuation of the suspected offending drug is indicated.
- Symptoms resulting from AIHA are indistinguishable from other causes of hemolysis.
- The DAT is the primary tool for diagnosing AIHA, but it may be negative in AIHA.
- Warm-antibody AIHA is treated with glucocorticoids, rituximab, other immunosuppressive medications, and splenectomy.
- Avoidance of cold environments may be sufficient to avoid complications of CAD. Rituximab and chemotherapy can control chronic hemolysis.
- Severe acute anemia from AIHA is lethal; survival may be measured in hours. Blood transfusion can be lifesaving. Involvement of transfusion medicine or blood bank may be needed to minimize delays caused by serologic incompatibility expected with AIHA. "Lack of compatible units" is not justification to forego transfusion.

Paroxysmal nocturnal hemoglobinuria

CLINICAL CASE

A 38-year-old man is admitted to the hospital for evaluation of severe throat pain. He has a history of PNH and received immunizations 4 days ago in preparation for starting eculizumab. On exam he is in pain, has slight scleral icterus, and the pharynx is hyperemic. Computerized tomography (CT) scan and ENT endoscopy are unrevealing. He is treated with broad-spectrum antibiotics, but his pain worsens and he requires a patient controlled analgesia pump (PCA). Laboratory studies are significant for a hemoglobin of 8.9 g/dL, down from his baseline of 10.8 g/dL with an absolute reticulocyte count of 350 × 109/L. White count and platelet count are normal. Indirect bilirubin is elevated at 3.7 mg/dL. LDH is increased at 750 U/L. DAT is negative. Prior PNH testing reveals a Type III clone of 42% on granulocytes.

Pathophysiology

PNH is an acquired, clonal hematopoietic stem cell disorder from mutations in the phosphatidylinositol glycan class A gene (*PIGA*) located on the X chromosome. The mutation causes deficiency of more than 150 glycosylphosphatidylinositol (GPI)-linked membrane proteins, but deficiency

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of CD55 and CD59 cause profound RBC sensitivity to complement-mediated lysis. Complement activation occurs by 3 pathways: classical (initiated by antibodies via C1q), lectin (initiated by microbial carbohydrate antigens via mannose-binding lectin), and alternative. The alternative pathway is active at baseline and functions in immune surveillance by a process called *tickover*. Tickover is spontaneous C3 hydrolysis that covalently labels nearby surfaces with C3b (Figure 8-9); nonself surfaces cannot arrest complement propagation and this leads to the formation of the terminal complement C5b9 membrane attack complex and cell lysis; human self surfaces contain complement regulators (CFH, CD55, CD59, CD46) that arrest complement amplification and hydrolyze C3b into inactive C3d. RBC lack CD46 and weakly interact with CFH via cell-surface sialic acid residues, and as a result RBCs without CD55 and CD59 are lysed by the spontaneous activity of the alternative pathway of complement. Because the alternative pathway also serves to amplify the classical and lectin pathways, acute hemolytic episodes in PNH can be triggered by infections, inflammation, surgery, etc. The stem cell *PIGA* mutation leads to the GPI protein defect in all cell lines, not just RBC. Although mutation of PIGA is necessary for the development of PNH, a PNH clone can be present without clinical features of PNH. Hemolysis and thrombosis are related to clone size, with hemolysis occurring with granulocyte clones of 20% or greater; the risk of thrombosis is significantly higher in patients with clone sizes 50% or greater. PNH clones may expand over time, regress, or remain stable. Current thinking suggests that the PNH clone arises by escape of immunologic attack of the bone marrow cells. Consistent with this, PNH clones are common in aplastic anemia and acquired bone marrow failure, where they are found in 20% to 59% of patients, but the clones are usually small (~10%).

Laboratory findings

PNH is a disorder of hemolysis: hemoglobin, LDH, and haptoglobin will track with the degree of hemolysis. Although PNH is a complement-mediated disorder, the DAT is negative because the PNH RBC are rapidly destroyed in the vasculature after initial complement deposition. If patients are on complement inhibitor therapy, C3 positive RBC are seen in the DAT because cell lysis is prevented by terminal complement inhibition. If aplastic anemia is present, other cytopenias may be observed. Bone marrow biopsy in patients with PNH (without bone marrow failure) may show erythroid hyperplasia. Iron deficiency can coexist, resulting from urinary losses of hemoglobin.

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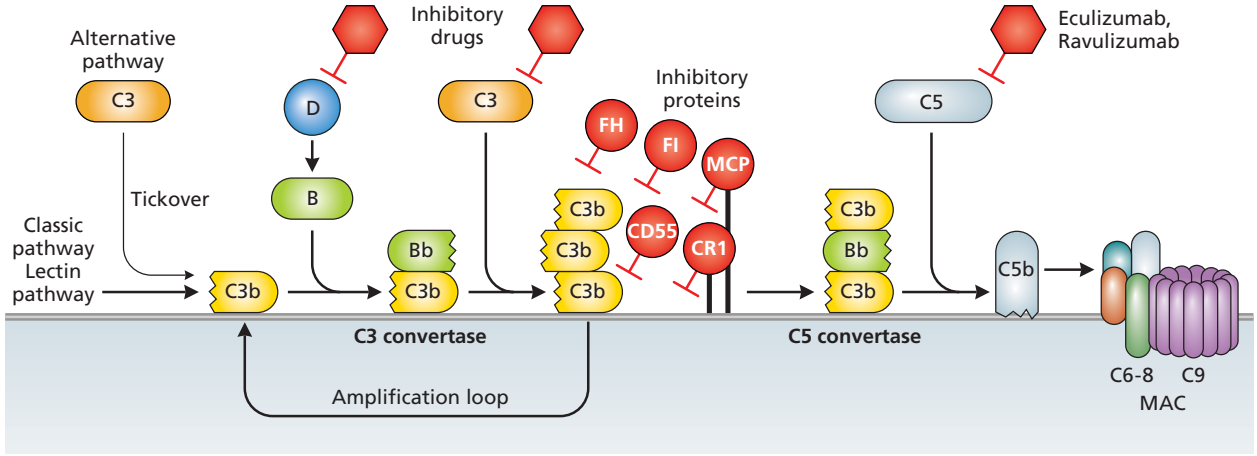
Figure 8-9 Complement cascade with inhibitors. CR1, membrane cofactor protein (MCP/CD46), factor I (FI), factor H (FH), and CD55 are the major proteins that inhibit the alternative pathway of complement (APC). Of these, FH is the most critical. FH is a fluid-phase protein that can bind to cells via its interaction with cell surface glycosaminoglycan and sialic acid residues. It inhibits the APC in the fluid phase and on the cell surface. It competes with factor B for C3b attachment, thereby limiting formation of the C3 convertase, and it accelerates decay of the C3 convertase. Deficiency or impaired function of FH leaves healthy host cells and tissues highly vulnerable to complement-mediated attack. Complement FI functions as a complement inhibitor in the presence of its cofactors (FH, MCP/CD46, and CR1), to cleave and inactivate C3b (in the alternative pathway) and C4b (in the classical pathway and lectin pathway), blocking C3 convertase formation. CD55 (also known as a decay-accelerating factor) is a cell surface glycosylphosphatidylinositol-anchored protein that inhibits the classical and alternative pathway C3 convertases and accelerates their decay. CD59 blocks terminal complement by preventing C9 from oligomerizing with the C5b-8 complex. MAC, membrane attack complex. Adapted from Merrill SA, Brodsky RA, *Hematology (Am Soc Hematol Educ Program)*. 2018;2018(1):371-376.



Laboratory diagnosis

Historically, PNH was diagnosed by observing increased lysis of RBC in acidified serum (Ham test), which activates the alternative pathway of complement and destroys PNH RBCs. In modern times, PNH is diagnosed by flow cytometry of peripheral blood. Flow cytometry is a robust diagnostic methodology that quantifies GPI-linked proteins with monoclonal antibodies against CD55 and CD59, and fluorescein-labeled aerolysin (FLAER), a bacterial toxin that recognizes GPI-linked proteins. Since the PIGA defect affects all cells produced by the bone marrow, patients can be diagnosed in a hemolytic crisis by detecting the GPI-deficient monocytes and neutrophils in the peripheral blood.

Stem cells expressing *PIGA* generate progeny with normal levels of GPI-linked proteins, called *Class I cells*; stem cells lacking PIGA cannot make GPI-linked proteins and produce Class III cells (lacking CD55 and CD59). Class II cells have detectable, but abnormally low, content of GPI-linked proteins; this may occur through microparticle transference as noted in vitro. Therefore, low amounts or absence of Class II and III red cells do not denote the absence of PNH. Detection of PNH granulocytes is not influenced by blood transfusion or hemolysis. PNH-nucleated cells are more resistant to lysis from complement, allowing their detection during periods of increased PNH hemolysis.

Clinical manifestations

The presentations of patients with PNH are surprisingly variable and may perplex clinicians. Anemia can be compensated and mild, or may be severe during active hemolysis. Despite the name, nocturnal hemoglobinuria is not common. Other cytopenias may be present from bone marrow failure. Careful consideration of PNH is warranted for patients with myelodysplasia (MDS) or aplastic anemia when hemolysis is observed. Acute hemolytic episodes may be associated with severe pain (chest pain, back pain, esophageal spasm, abdominal pain) or erectile dysfunction. Acute hemolysis can be precipitated by infection, surgery/trauma, immunizations, pregnancy, and any inflammatory condition (such as gout) where complement levels increase or complement activation occurs. Given the proclivity for thrombosis, when patients present with symptoms it is helpful to assess for possible thrombotic causes (ie, if abdominal pain, consider portal or hepatic vein thrombosis).

Patients frequently have thrombotic complications that can be life-threatening and may represent the initial manifestation of PNH. In addition to venous thrombosis involving an extremity, there is a propensity for thrombosis of unusual sites such as hepatic veins (Budd-Chiari syndrome), other intra-abdominal veins, cerebral veins, and venous sinuses. Thus, complaints of abdominal pain or severe headache should alert clinicians to consider thrombosis in patients with PNH. The thrombotic tendency is particularly enhanced during pregnancy or surgery. Although complement and coagulation are interconnected, PNH patients may have recurring thromboses despite adequate anticoagulation, however therapy with eculizumab can prevent recurrent thrombosis.

Treatment

For patients who have mild hemolysis and are asymptomatic (usually clone size <10%), no clinical intervention is needed. Folate supplementation is generally recommended regardless of whether a treatment is indicated or not. Because expansion of the clone may occur, the size of the clone may be monitored every 12 months. The most effective therapies are complement inhibition with eculizumab or ravulizumab. Initiation of eculizumab is indicated in patients with thrombosis, clinically relevant hemolytic anemia, or symptoms attributable to hemolysis.

Allogeneic hematopoietic stem cell transplantation is the only cure for PNH. However, because of the high risk for serious complications including death, it should not be offered as the initial treatment. Rather, it should be reserved for patients with no access to complement inhibition, those with severe aplastic anemia, or the rare individuals whose hemolysis or thrombosis is not adequately controlled by complement inhibition. For patients with PNH and marrow failure who lack an HLA-matched sibling donor, immunosuppressive therapy may be attempted.

Eculizumab is approved in PNH to treat hemolysis based on efficacy in phase 3 clinical trials. Eculizumab reduces intravascular but not extravascular hemolysis, eliminates or reduces transfusion requirement in most patients (but not all), improves quality of life, ameliorates pulmonary hypertension, and decreases the risk of thrombosis. Patients may require dose escalation to control hemolysis, especially after surgery or during pregnancy. Ravulizumab is also approved for PNH, it is an extended half-life molecule with changes enabling endosomal recycling. Ravulizumab can be administered every 8 weeks, which is beneficial for patient adherence and convenience. Ravulizumab can reduce residual hemolysis for patients on eculizumab, and 93% of patients prefer ravulizumab compared to eculizumab because of convenience and quality of life improvement. However, neither eculizumab nor ravulizumab treat the

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marrow failure in aplastic anemia or the underlying cause of PNH and must be used continually unless the PNH clone recedes below a point of clinical relevance, which is uncommon.

Although eculizumab and ravulizumab are generally well-tolerated, the most serious complication is sepsis caused by *Neisseria* organisms, and both therapies have Risk Evaluation and Mitigation Strategies (REMS) programs. Patients congenitally lacking one of the terminal complement components, C5 to C9, are known to be at risk for *Neisseria* infection, and patients receiving terminal complement inhibitors are at risk. Vaccination against *Neisseria meningitidis* is recommended 2 weeks before starting therapy, but vaccination itself can provoke a hemolytic episode. Patients can still be susceptible to serotypes not included in vaccinations (Non-ACWY, Non-B). Because vaccination does not eliminate the risk completely, patients are provided with notification cards and education to seek medical care if they have symptoms potentially consistent with *Neisseria* infection. Antibiotic prophylaxis is necessary if complement inhibition has to be administered less than 2 weeks after vaccination.

Thrombosis is the leading cause of death in PNH patients. It should be treated promptly with anticoagulation. Prophylactic anticoagulation is controversial and may not be necessary. In one large, nonrandomized trial, primary prophylaxis with warfarin decreased the risk of thrombosis in patients with large PNH clones (>50% PNH granulocytes). Because eculizumab decreases the risk of thrombosis and ameliorates the hypercoagulable phenotype seen in PNH, prophylactic anticoagulation is not indicated for patients on complement inhibitor therapy. Patients with thrombosis may not require long-term anticoagulation if treated with complement inhibition. The question remains: is prophylactic anticoagulation needed in patients who do not require complement inhibition? Pregnant/postpartum patients and those having surgery are at increased risk of thrombosis generally, in the presence of PNH these patients are expected to have further increased thrombotic risk and would likely benefit from prophylactic anticoagulation. Eculizumab crosses the placenta and is present in cord blood. However, its use during pregnancy is apparently safe and appears to reduce fetal mortality and maternal morbidity. The safety of ravulizumab in pregnancy has not been examined.

Prognosis

Before eculizumab, the median survival for PNH was 10 to 15 years. In the posteculizumab era, there is minimal to no difference in survival compared to age-matched healthy controls. However, patients with underlying aplastic anemia or MDS have decreased survival.

CLINICAL CASE *(continued)*

The patient received vaccinations to begin eculizumab in the near future to control hemolysis. His DAT is negative because although hemolysis is increased from baseline, the C3b-positive RBC are quickly destroyed. Immunization, surgery, pregnancy, or trauma can cause complement activation, and an acute flare of PNH hemolysis. Flares can present unusually with thrombosis, pain, or icterus/jaundice. This patient's throat pain was related to hemolysis; eculizumab was started and his pain resolved. PCA was discontinued approximately 12 hours later.

KEY POINTS

- PNH is an acquired clonal hematopoietic stem cell disorder caused by a somatic mutation of the *PIGA* gene that results in hematopoietic cells lacking GPI-linked proteins.
- Patients may experience chronic hemolytic anemia, cytopenias, pain paroxysms, or a thrombotic tendency.
- Flow cytometry to identify cells lacking GPI (FLAER) or GPI-linked proteins (CD55 and CD59) are the standard diagnostic tests.
- PNH clones have been identified in individuals without hematologic abnormalities.
- Eculizumab and ravulizumab, monoclonal antibodies directed against C5, eliminate or reduce hemolysis, improve quality of life, and decrease the risk of thrombosis.
- *Neisseria* sepsis is a potentially fatal complication of C5 complement inhibition. Vaccination against *Neisseria* should be given 2 weeks before initiation of complement inhibition. Antibiotic prophylaxis is necessary if anticomplement therapy has to be administered less than 2 weeks after vaccination.
- Prompt evaluation is indicated for symptoms of thrombosis, particularly at unusual sites. Anticoagulation is indicated for documented thrombosis, but long-term anticoagulation may not be needed after initiation of complement inhibition therapy.
- Allogeneic hematopoietic cell transplantation has curative potential. Because of the risk of serious or fatal complications, its use should be reserved for those patients with severe cytopenias or patients with severe hemolysis or thrombosis refractory to complement inhibition.

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Fragmentation hemolysis

CLINICAL CASE

A 59-year-old man is referred for evaluation of anemia and thrombocytopenia. His past history is significant for an elevated ferritin (1649 ng/mL) treated with phlebotomy (liver biopsy with normal iron content before phlebotomy). He reports 2 years of intermittent spells of fever, nausea, fatigue, back pain, dark urine, and erectile dysfunction. On examination, there are no abnormalities. Laboratory data are significant for a hemoglobin of 10.9 g/dL (normal 6 months prior), platelets 125 (prior normal), LDH 465 U/L, and total bilirubin 6.5 mg/dL. Creatinine is 1.8 mg/dL from prior normal, urinalysis reveals numerous RBCs. Examination of the blood smear reveals rare schistocytes. DAT is negative.

Fragmentation hemolysis takes place within the vasculature. Features characteristic of intravascular as opposed to extravascular hemolysis include the presence of free hemoglobin in the plasma and urine, resulting in red urine and pink plasma. If the hemolysis is chronic, urine hemosiderin may be present. In fragmentation hemolysis, schistocytes are a prominent feature of the blood smear (Figure 8-10). The differential diagnosis of fragmentation hemolysis is summarized in Table 8-8.

Pathophysiology

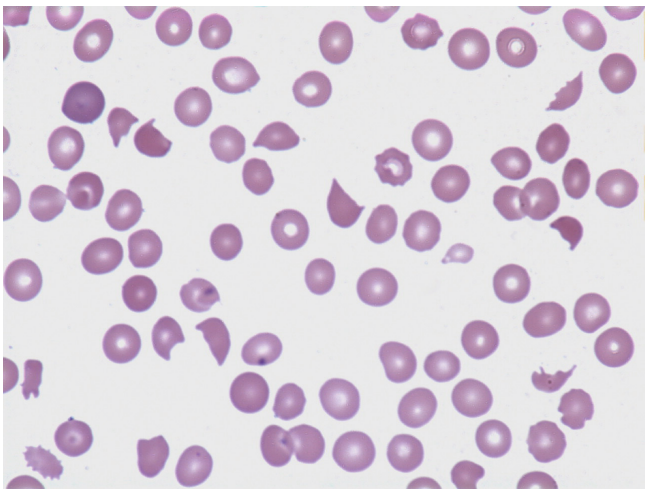
Among the several causes of fragmentation hemolysis, the common thread is mechanical damage to RBCs, producing fragmented RBCs or schistocytes on the blood smear. When microvascular or endothelial injury is present, the process is termed microangiopathic hemolytic anemia (MAHA). When thrombosis or microthrombi are present, the term *thrombotic microangiopathy* (TMA) is used. TMA is a family of disorders that present similarly. One mechanistic archetype is TTP; ADAMTS13 deficiency causes abnormal processing of von Willebrand factor leading to platelet activation. On the other end of the spectrum is Shiga toxin–mediated TMA (ST-TMA, formerly known as hemolytic uremic syndrome), where a bacterial toxin causes endothelial damage and organ dysfunction. Between these 2 pathophysiologic archetypes reside other TMAs, such as disseminated intravascular coagulation (DIC) and complement-mediated TMA (CM-TMA, formerly *atypical hemolytic uremic syndrome*). CM-TMA is driven by complement, however complement has also been implicated in ST-TMA, as well as in other disorders previously thought to be distinct (malignant hypertension, lupus nephritis TMA). Further changes to the classification system of CM-TMA will likely occur over time, but currently CM-TMA are considered as either primary (idiopathic or inherited) or triggered (by an underlying condition such as infection, lupus, etc.)

Injury to blood vessel endothelium, thrombin activation, and platelet activation all result in formation of fibrin strands in the circulation. The shearing force generated as the RBCs pass through the fibrin strands causes the RBCs to be fragmented into small, irregular pieces. RBCs may be broken into pieces by direct mechanical trauma as with a dysfunctional mechanical heart valve in which high-velocity jets of blood strike a nonendothelialized surface. The resulting small RBC fragments are self-sealing and continue to circulate, albeit with shortened survival. This is caused, in part, by their decreased deformability, which results in accelerated removal by the spleen.

Table 8-8 Differential diagnosis of fragmentation hemolysis

Complement-mediated thrombotic microangiopathy
Cardiac valve disease
Disseminated intravascular coagulation
Hemolysis, elevated liver enzymes, low platelets (HELLP) syndrome
Shiga toxin thrombotic microangiopathy
Malignancy
Malignant hypertension
Scleroderma renal crisis
Thrombotic thrombocytopenia purpura
Vasculitis

Figure 8-10 Schistocytes. Source: ASH Image Bank/Peter Maslak (image 00003718).



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Etiology

Cardiac valve hemolysis

Hemolysis may occur with calcific or stenotic native heart valves. In the absence of severe valve disease this is usually mild and compensated. Mechanical heart valves have a smaller diameter than the native heart valves and valve dysfunction or perivalvular regurgitation may result in intravascular hemolysis. An aged or damaged valve surface may become irregular, leading to thrombus formation. In a high-flow state, such as exists across the aortic valve or across a regurgitant mitral valve, the formation of jets and turbulent flow results in high shear stress that may exceed the stress resistance of the normal RBC. Hemolysis may be made worse with concomitant cardiac failure or high-output states. Bioprosthetic valves demonstrate less hemolysis than prosthetic valves, and larger mechanical vales have less hemolysis than smaller valves. Transcatheter aortic valve replacement/implantation (TAVR or TAVI) can also cause hemolysis, and the incidence ranges from 9% to 26%.

Ruptured chordae tendineae, aortic aneurysm, and patch repair of cardiac defects, as well as intraventricular assist devices and aortic balloon pumps used in the management of severe heart failure, have been associated with traumatic hemolysis. Schistocytes are usually observed but may not be abundant. Therapy ideally addresses the valvular abnormality, however addressing possible concomitant causes of anemia can be helpful, such as iron deficiency or chronic kidney disease.

Thrombotic thrombocytopenic purpura

TTP occurs because of platelet activation by abnormally high molecular weight von Willebrand factor multimers, which accumulate from either acquired or congenital deficiency of ADAMTS13. TTP may be present similarly to CM-TMA; ST-TMA; hemolysis, elevated liver enzymes, low platelets (HELLP) syndrome; acute fatty liver of pregnancy (AFLP); and streptococcus pneumoniae-TMA. Recent advances in TTP include upfront use of rituximab, preventative rituximab to prevent relapse with dropping ADAMTS13 activity, and disruption of the VWF-GP1b interaction with caplicizumab it must be emphasized that patients rarely have the pentad of symptoms associated with the disease (fever, renal disease, anemia, thrombocytopenia, neurologic findings).

Drug-induced TMA

Drug-induced TMA can be caused by mitomycin, gemcitabine, tacrolimus, and sirolimus, and even quinine found in tonic water. An important recent addition is the TMA observed in hemophilia patients treated with emicizumab and high doses of factor VIII inhibitor bypassing activity (FEIBA). Proposed mechanisms involve toxic endothelial injury, and immune-mediated reactions. Patients often have a presentation similar to CM-TMA; however, consideration of TTP is warranted. Therapy involves cessation of the offending drug and supportive care, however eculizumab and plasma exchange have been used.

Complement-mediated TMA

CM-TMA (formerly known as *atypical hemolytic uremic syndrome*) is more common in adults than ST-TMA (formerly *HUS*). CM-TMA is a disorder of predominating renal failure/impairment with intravascular thrombi, often with hemolytic anemia and thrombocytopenia; in contrast, TTP is a disorder of thrombocytopenia with hemolytic anemia, and minor (or no) renal impairment. It must be noted that 15% to 16% of children and adults with CM-TMA have a normal platelet count at presentation. CM-TMA may develop from underlying defects in complement regulation; however, many adults have no known underlying causal genetic predisposition. The alternative pathway of complement has been shown to be abnormally active in patients with CM-TMA. Complement inhibition with eculizumab or ravulizumab are approved therapies and are preferred to plasma exchange because of superior responses and long-term outcomes. Complement inhibition can arrest renal damage, allow liberation from dialysis, and prevent clinical deterioration into critical illness from CM-TMA. It must be noted that complement inhibition is not a "one-dose-fits-all therapy" despite the current FDA approved dosing schema; patients may require higher doses if cytopenias and hemolysis do not improve. Although plasma exchange was used in the precomplement inhibition era, long-term outcomes were poor, and early use of complement inhibition leads to statistically significant improvements in renal function. CM-TMA is often categorized as primary or secondary to an underlying trigger. Successful identification of primary versus trigger can be difficult, such as in malignant hypertension, where CM-TMA can cause hypertension and hypertension can cause CM-TMA; CM-TMA from many trigger etiologies responds to complement inhibition.

Shiga toxin–mediated TMA

ST-TMA is a TMA precipitated by Shiga toxin, most commonly in the setting of *Escherichia coli* infection, but *Shigella*, *Citrobacter*, and *Salmonella* can express the toxin. ST-TMA and CM-TMA are clinically indistinguishable, but

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patients with ST-TMA may have an antecedent diarrheal illness and may note bloody diarrhea. While large outbreaks can occur, most cases of ST-TMA are sporadic. Shiga toxin causes endothelial cell damage, and several lines of evidence implicate complement activation in ST-TMA including increased C3 cleavage products, depressed C3 correlating with renal impairment, acute elevations of the soluble C5b9 terminal complement complex, and demonstration of alternative pathway activation in the acute phase of illness. Shiga toxin may activate complement by directly binding to the major soluble complement inhibitor, Complement Factor H, or by interactions with leukocytes and platelets. Although supportive care and plasma exchange have been used in ST-TMA, recent experiences suggest complement inhibition with eculizumab may have a role in severe disease, neurologic impairment, and in patients refractory to conventional therapy.

Disseminated intravascular coagulation

Disseminated intravascular coagulation is associated with many underlying disorders, including sepsis, vascular malformations, obstetrical complications, and malignancy. The disorder is characterized by activation of coagulation and generation of excess thrombin leading to deposition of fibrin strands in arterioles, venules, and capillaries. MAHA may be present, but often is not severe enough to cause morbidity. Advanced malignancy presents with MAHA and DIC in approximately 7% of cases. The presence of DIC is a poor prognostic finding in solid tumor patients. Mucin-producing adenocarcinomas are frequent etiologies, such as lung, pancreatic, and gastric adenocarcinomas. Promyelocytic leukemia characteristically presents with DIC due, at least in part, to the release of tissue factor from promyelocytic granules. If treatment is effective at reversing the underlying condition causing DIC, hemolysis and the coagulopathy often resolve. In DIC hemorrhage is common, as well as thrombosis. Unfractionated heparin (500 IU/h flat infusion, or aPTT guided infusion) and low-molecular weight heparin have been used to control DIC, and this approach can facilitate DIC trigger identification and therapy of the underlying cause.

HELLP syndrome

HELLP syndrome, which is a serious complication of late pregnancy, is part of a spectrum including preeclampsia. Thrombocytopenia and MAHA with or without renal failure may also occur in pregnancy because of TTP, CM-TMA, and AFLP. It is important to distinguish TTP, CM-TMA, and AFLP from HELLP and preeclampsia for therapeutic reasons. It is important to note that HELLP can present postpartum. HELLP is discussed in depth in Chapter 3.

Kasabach-Merritt syndrome

Kasabach-Merritt syndrome is characterized by consumptive coagulopathy occurring in the capillaries of a large kaposiform hemangioendothelioma. MAHA accompanies evidence of DIC. A number of treatments, including glucocorticoids, chemotherapy, interferon-alfa, embolization, and surgical removal have been tried with some success.

Foot strike hemolysis

Foot strike hemolysis, also known as *march hemoglobinuria*, has been described in soldiers subjected to long foot-stomping marches in rigid-soled boots, long-distance runners, conga drummers, pneumatic hammer operators, and karate enthusiasts. Hemoglobinuria occurs shortly after the episode of exercise. The hemolysis is caused by direct trauma to RBCs in the blood vessels of the extremities. This condition has become much less common as shoe technology has improved. Cessation of the activity always leads to resolution of the hemolysis.

CLINICAL CASE *(continued)*

Extensive testing for hematologic or malignant causes of MAHA was negative, including bone marrow biopsy. Ultimately, he was diagnosed with Antineutrophil cytoplasmic antibody vasculitis by renal biopsy. Serum myeloperoxidase antibodies were positive. He was treated with corticosteroids and rituximab for vasculitis, the MAHA resolved, and his ferritin normalized.

Hemolytic anemia caused by chemical or physical agents

CLINICAL CASE

A 23-year-old woman is referred for evaluation of mild anemia noted during a workup of liver function test abnormalities. Her recent history has been significant for bizarre schizophrenia-like behavior and arthritis. She has not had a menstrual period in several months. Recent slit-lamp examination by an ophthalmologist revealed golden brown pigmentation of the cornea. Physical examination is otherwise unremarkable. Laboratory data suggest a DAT-negative hemolytic anemia. Liver enzymes are moderately elevated. A ceruloplasmin level returns low at 110 µmol/L.

The use of dapsone to prevent or treat *Pneumocystis jirovecii* in patients with HIV/AIDS, bone marrow or organ transplantation, and chronic corticosteroids is common. Such drugs may cause methemoglobinemia and oxidative hemolysis in normal patients or those with G6PD deficiency.

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Ribavirin, previously used to treat hepatitis C virus infection, is a frequent cause of hemolysis by an unknown mechanism. The hemolysis is dose dependent and decreases or resolves with decreased ribavirin dose or discontinuation of the drug. The rate of sustained HCV response, however, also decreases with dose reduction. Erythropoietin has been used as an adjunct to maintain ribavirin therapy at full dose.

Phenazopyridine is a bladder analgesic that is used to treat the symptoms of cystitis. In high doses, it has been associated with oxidative hemolysis. It is recommended that patients be treated for no more than 2 days. Overdoses, prolonged administration, and renal insufficiency have led to methemoglobinemia and severe hemolysis, occasionally severe enough to induce acute renal failure.

Lead intoxication can lead to a modest shortening of RBC life span, although the anemia more often is caused by an abnormal heme synthesis and decreased production of erythrocytes. On the blood smear, RBCs are normocytic and hypochromic, with prominent basophilic stippling in young polychromatophilic cells.

Copper causes hemolysis through direct toxic effects on RBCs and has been observed in association with hemodialysis. Copper accumulates in RBCs and disrupts normal metabolic function through a variety of mechanisms, including oxidation of intracellular reduced glutathione, hemoglobin, and NADPH and inhibition of multiple cytoplasmic enzymes. Wilson disease, caused by a mutation of the ATP7B gene, leads to absence or dysfunction of a copper-transporting ATPase encoded on chromosome 13. This subsequently results in lifelong copper accumulation. Hemolytic anemia may be an early manifestation. The hemolytic process in Wilson disease varies in severity and duration. Kayser-Fleischer rings caused by the deposition of copper around the periphery of the cornea may be present, but this has poor sensitivity compared to assays of urinary copper excretion or ceruloplasmin. Treatment consists of penicillamine, which mobilizes copper stores. Acute hemolysis in Wilson disease has been treated successfully with plasmapheresis.

Certain spider bites may be associated with traumatic RBC fragmentation. In the southern United States, the brown recluse spider (*Loxosceles reclusa*) is the most common species causing hemolysis. The toxin proteolyzes the RBC membrane through damage to protein band 3 and other integral proteins. In the northwestern United States, hemolysis has been noted after hobo spider (*Tegenaria agrestis*) bites. Microangiopathic hemolysis may occur after the bite of pit vipers (eg, rattlesnakes, cottonmouth moccasins, and copperheads) associated with DIC induced by the venom. Cobra venom contains phospholipases that may cause hemolysis. Massive bee and wasp stings rarely have been associated with intravascular hemolysis.

Fragmentation hemolysis has been described after injury from a variety of physical agents. Thermal injury can lead to severe intravascular hemolysis. This is best described in patients suffering from extensive third-degree burns. At temperatures above 47°C, irreversible injury occurs to the RBC membrane. Shortened RBC survival has been noted after ionizing radiation exposure. Patients treated with hypotonic bladder irrigation may also develop brisk hemolysis.

CLINICAL CASE *(continued)*

The patient presented in this section displays the classic historical and physical findings of Wilson disease. The low ceruloplasmin level is diagnostic. Hemolytic anemia has been well-described in this disease. Once the severity of her liver disease is further evaluated, treatment with penicillamine should be considered. The hemolytic anemia is likely to resolve as excess copper is removed.

Hemolytic anemia caused by infection

CLINICAL CASE

A 21-year-old man went to the emergency department complaining of fever and shaking chills. He was home 2 weeks, following a 6-month deployment in eastern Afghanistan with the United States Army.

He regularly took mefloquine 250 mg weekly while in Afghanistan. He was instructed to continue the weekly mefloquine for 4 more doses postdeployment, plus primaquine 15 mg daily for the first 2 weeks. On examination, he appeared acutely ill. His vital signs were blood pressure of 126/66, pulse 110, respiration 20, and temperature 39°C. The remainder of the examination was unremarkable. There was no splenomegaly. A Wright-Giemsa–stained thick blood smear confirmed the diagnosis of Plasmodium vivax malaria.

Infection may lead to hemolysis through a variety of mechanisms. Parasites may directly invade RBCs, leading to premature removal by macrophages of the liver and spleen. Alternatively, hemolytic toxins may be produced by the organism and lead to damage of the RBC membrane. Development of antibodies to RBC surface antigens has been well-described with certain viral and bacterial illness, especially infectious mononucleosis and *Mycoplasma pneumoniae* infections. Hypersplenism may ensue, which can further decrease RBC life expectancy. In addition, the

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antibiotic drugs used to treat a variety of these infections may lead to further hemolysis in G6PD-deficient individuals. Anemia that occurs with concomitant acute or chronic infection is likely to be multifactorial, with the anemia of chronic inflammation often coexisting and predominating.

RBC membrane injury caused by bacteria

Clostridial sepsis

Sepsis from *Clostridium perfringens* or *Clostridium septicum* is seen in patients with anaerobic subcutaneous infections, in body areas of impaired circulation, after trauma, after septic abortion or postpartum sepsis, and in patients with acute cholecystitis with gangrene of the gallbladder or bowel necrosis. It has a mortality rate of almost 75%. Severe neutropenia is a risk factor. The α toxin of *Clostridium* is a lecithinase (phospholipase C) that disrupts the lipid bilayer structure of the RBC membrane, leading to membrane loss and massive hemolysis. A common clinical scenario is the inability of the phlebotomist in the emergency room to draw a nonhemolyzed blood sample despite multiple attempts. The plasma may be a brilliant red color, and there may be dissociation between the hemoglobin and hematocrit values because of the plasma hemoglobin levels reaching several grams per deciliter. Acute renal failure, secondary to excessive hemoglobinuria, may ensue. Renal failure and hepatic failure contribute to the high mortality in clostridial sepsis.

Hemolytic anemias with gram-positive and gram-negative organisms

Septicemia and endocarditis caused by gram-positive bacteria, such as streptococci, staphylococci, *Streptococcus pneumoniae*, and *Enterococcus faecalis* are often associated with hemolytic anemia. The anemia in patients with infections caused by gram-positive cocci appears to result from the direct toxic effect of a bacterial product on erythrocytes. *Salmonella typhi* infection may be accompanied by severe hemolytic anemia with hemoglobinemia. In typhoid fever, the onset of hemolysis may occur during the first 3 weeks of illness, with anemia lasting from several days to 1 week. *Salmonella* and other microorganisms can cause direct agglutination of RBCs in vitro, but it is not known whether this phenomenon contributes to in vivo hemolysis. In approximately 1/3 of patients with typhoid fever, a positive DAT develops, but hemolytic anemia is not manifest in all cases.

Immune hemolysis associated with infections

Pneumonia caused by *Mycoplasma pneumoniae* can be associated with production of cold agglutinins, IgM antibodies directed against the RBC I antigen. Hemolytic anemia associated with *Mycoplasma* may occur during the second or third week of the illness. The onset of the hemolysis may be rapid, usually occurring after recovery from respiratory symptoms. The clinical presentation often includes dyspnea or fatigue and the presence of pallor and jaundice. The blood smear shows RBC agglutination with or without spherocytosis and with polychromatophilia (Figure 8-7). Cold agglutinin titers at the onset of hemolysis usually exceed 1:256 and may reach higher levels, although they are typically lower than in cold agglutinin disease. The DAT is positive for complement deposition on RBCs. The hemolytic anemia associated with *Mycoplasma* pneumonia is self-limited, transient, and usually mild, although severe cases requiring corticosteroid therapy or plasmapheresis have been reported.

Infectious mononucleosis caused by Epstein-Barr virus infection may be associated with hemolytic anemia caused by cold agglutination. The cold agglutinin in this case is an IgM antibody directed against the i antigen. Severe hemolytic anemia associated with infectious mononucleosis is unusual, although anti-i antibodies frequently are present. When hemolytic anemia occurs, the mechanism involves fixation of complement on the RBC membrane by IgM antibodies. Hemolysis can occur by phagocytosis of RBC or by complement-mediated lysis.

Several other viral infections have been associated with AIHA. These include cytomegalovirus, herpes simplex, rubeola, varicella, influenza A, and HIV. Postviral acute hemolytic anemia in children may be caused by PCH. Patients with either congenital or tertiary syphilis may also develop PCH. Whereas PCH used to be fairly common in the late 19th and earlier 20th centuries, it is rare in the 21st century because of the disappearance of congenital and tertiary syphilis.

MAHAs associated with infection include bacteremia with gram-negative organisms, staphylococci, meningococci, and pneumococci, all of which can lead to DIC with endothelial damage and fibrin thrombi within the microcirculation. RBC injury results from mechanical fragmentation by fibrin strands in the vasculature. Microvascular damage induced by meningococcal and rickettsial infections (eg, Rocky Mountain spotted fever) may be associated with DIC, thrombocytopenia, and microvascular thrombi.

Hemolytic anemia associated with parasitic infestation of RBCs

Malaria

Malaria is the most common cause of hemolytic anemia worldwide. Transmitted by the bite of an infected female *Anopheles* mosquito, sporozoites that are injected from the mosquito make their way to liver

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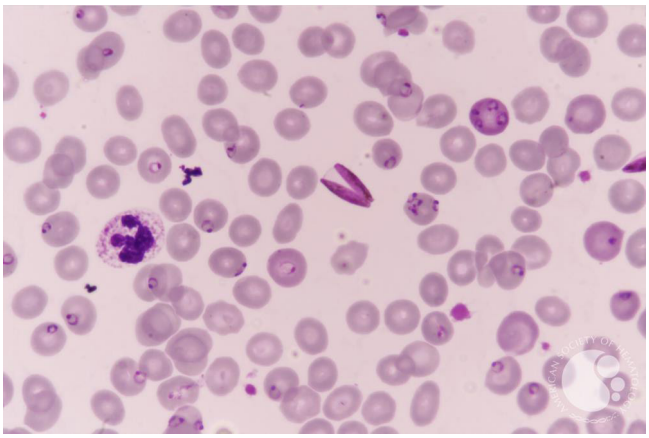
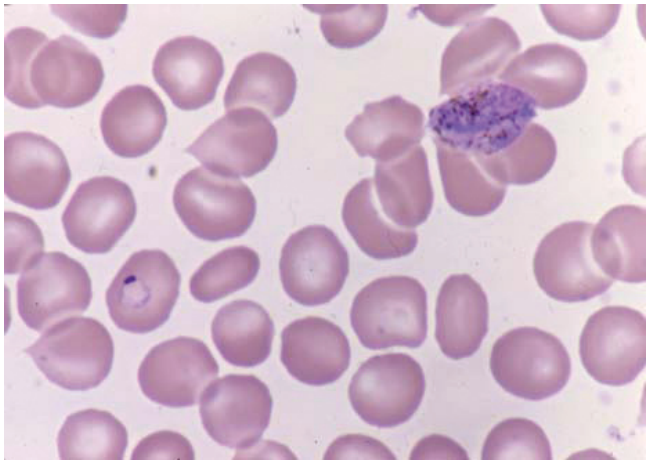


Figure 8-11 The intraerythrocyte parasite *Plasmodium falciparum*. Source: ASH Image Bank/Behzad Poopak and Sina Zargaran (image 00061470)

cells. Merozoites enter into the bloodstream 1 to 2 weeks later. Hemolysis in malaria results directly from RBC infestation by *Plasmodium* organisms (Figures 8-11 and 8-12). Noninfected RBCs may be hemolyzed by poorly understood mechanisms. Infested RBCs are selectively removed from the circulation by the spleen, with some RBCs reentering circulation after splenic pitting of parasites. Previously infested RBCs manifest membrane and metabolic abnormalities along with decreased deformability. In addition, the *Plasmodium* species digests the host RBC hemoglobin for its own use as a nutrient.

The severity of the hemolytic process is often out of proportion to the degree of parasitemia. *Plasmodium vivax* and *Plasmodium ovale* invade only reticulocytes, whereas *Plasmodium malariae* invades only mature erythrocytes.

Figure 8-12 The intraerythrocyte parasite *Plasmodium vivax:* trophozoite (ring form) and female gametocyte.



Plasmodium falciparum invades erythrocytes of all ages and is associated with more severe hemolysis. In areas where malaria has been a frequent cause of death for many centuries, a number of genetic polymorphisms are prevalent, including G6PD deficiencies, thalassemias, and hemoglobinopathies. These polymorphisms have in common the ability to interfere with the ability of the malaria parasites to invade RBCs.

With *Plasmodium falciparum* infection, intravascular hemolysis may be severe and associated with hemoglobinuria (blackwater fever). Another potentially lethal complication of *Plasmodium falciparum* infection, cerebral malaria, results from expression of a combination of parasite-induced RBC surface proteins including *Plasmodium falciparum* erythrocyte protein 1. These RBCs adhere to receptors on vascular endothelium in various organs, including the central nervous system, resulting in vaso-occlusion and neurologic manifestations.

Diagnosis of malaria is based on identification of parasite-infected RBCs on a thick Wright-stained blood smear. The distinction of *Plasmodium falciparum* infection from the other species is important because its treatment may constitute a medical emergency. The findings of 2 or more parasites per RBC and infestation of >5% of RBCs are characteristic of *Plasmodium falciparum* infection.

Chemoprophylaxis should be offered to all people planning travel to known areas of endemicity. The hemolytic anemia of malaria resolves after successful therapy with quinine, chloroquine, artemisinin, and other drugs, depending on the species of malaria. Many of these agents may be associated with drug-induced hemolysis in patients with G6PD deficiency.

Babesiosis

Babesiosis is a protozoan infection caused by *Babesia microti*. Once thought to be rare, outbreaks have been described with increasing frequency on Nantucket Island and in Cape Cod, northern California, and several other North American locations. The organism is transmitted by the bite of the *Ixodes* tick, which infects many species of wild birds and domestic animals and occasionally humans. Babesiosis rarely may be transmitted by transfusion with fresh or frozen-thawed RBCs. Infection leads to a clinical syndrome of fever, lethargy, malaise, and hemoglobinuria 1 to 4 weeks after the bite. Hemolytic anemia caused by intravascular hemolysis occurs, and renal and liver function tests are frequently abnormal. The disease is often asymptomatic in people with intact spleens; patients who have undergone splenectomy are at high risk for severe symptomatic infection.

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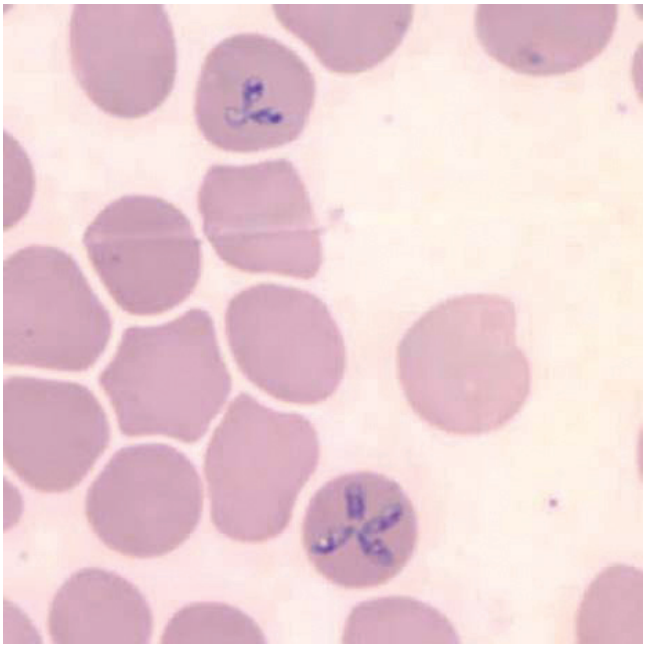

Figure 8-13 The intraerythrocyte parasite *Babesia microti* (Maltese cross formation).

Babesia infection can be diagnosed by demonstrating typical intraerythrocytic parasites on a thin blood smear (Figure 8-13). The standard treatment is atovaquone plus azithromycin. A warm AIHA can manifest 2 to 4 weeks after diagnosis and may require immunosuppressive treatment. With the expanding range of *Ixodes* ticks, *Babesia* is a growing concern for patients who receive blood products.

Bartonellosis

Bartonellosis, caused by *Bartonella bacilliformis*, manifests in 2 clinical stages: an acute hemolytic anemia and a chronic granulomatous phase. The microorganism enters the blood following the bite of an infected sand fly. The infective *Bartonella* agent adheres to the membrane of RBCs that are then removed by the spleen. The hemolytic anemia of bartonellosis develops rapidly and may be severe, with hemoglobinemia and hemoglobinuria. When untreated, this disorder is associated with high mortality. Survivors manifest a second stage of the disease with cutaneous granulomas. Bartonellosis is common in South America and has been reported in the Peruvian Andes and parts of Brazil, where it is also known as *Oroya fever*. On Giemsa-stained blood films, red-violet rods of varying lengths can be identified on RBCs and represent the bacteria. Effective treatment exists and consists of penicillin, streptomycin, chloramphenicol, or tetracycline.

CLINICAL CASE *(continued)*

The patient was admitted for treatment. The Centers for Disease Control and Prevention Malaria Hotline (1-770-488-7788) was called, and the regimen of chloroquine and primaquine was recommended for vivax malaria acquired in Afghanistan. He made a full recovery. He ultimately admitted that he had forgotten to take his prophylactic medications after leaving Afghanistan. The most common cause of failure of malaria prophylaxis in military or civilian populations is noncompliance.

Hemolysis from other causes

Several other conditions have been described to be associated with hemolytic anemia. These are generally of rare occurrence. Vitamin B_{12} deficiency rarely (1%) presents as hemolytic anemia, but usually with concomitant thrombocytopenia or neutropenia and may mimic TTP. Ineffective marrow erythropoiesis and pronounced hyperhomocysteinemia have been postulated to be potential causes of hemolysis. In patients with liver failure, biochemical changes may affect the integrity of the red cell membrane either structurally or metabolically, leading to premature RBC destruction, called spur-cell anemia. Iatrogenic causes such as administration of intravenous immunoglobulin and anti-D may lead to hemolysis, but they are self-limited and frequently mild. Congenital dyserythropoietic anemia types II and III also present with hemolytic anemia. They are extremely rare bone marrow failure syndromes characterized by failure of terminal erythropoiesis. Congenital dyserythropoietic anemia is covered in Chapter 15.

KEY POINTS

- RBC fragmentation syndromes are diverse in etiology.
- In all suspected cases of hemolytic anemia, the blood smear should be examined carefully for schistocytes. Their presence can direct differential diagnosis.
- RBC destruction can be at the macrovascular or microvascular (microangiopathic) level of the circulatory system. Classic examples include heart valve hemolysis, DIC, and TTP.
- Various chemical exposures or physical agents can cause fragmentation hemolysis.
- Infection can cause accelerated RBC destruction through a variety of mechanisms, including direct invasion, toxin production, and immune mechanisms.
- Malaria, the most common infectious disease worldwide, causes hemolysis through both direct parasitic invasion of RBCs and alterations in noninfected cells. It can be diagnosed by thorough review of a thick Wright-stained peripheral blood film.

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